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ORIGINAL RESEARCH

Association Between the Gut Microbiota and Blood Pressure in a Population Cohort of 6953 Individuals

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BACKGROUND: Several small-scale animal studies have suggested that gut microbiota and blood pressure (BP) are linked. However, results from human studies remain scarce and conflicting. We wanted to elucidate the multivariable-adjusted association between gut metagenome and BP in a large, representative, well-phenotyped population sample. We performed a focused analysis to examine the previously reported inverse associations between sodium intake and *Lactobacillus* abundance and between *Lactobacillus* abundance and BP.

METHODS AND RESULTS: We studied a population sample of 6953 Finns aged 25 to 74 years (mean age, 49.2±12.9 years; 54.9% women). The participants underwent a health examination, which included BP measurement, stool collection, and 24-hour urine sampling (N=829). Gut microbiota was analyzed using shallow shotgun metagenome sequencing. In age- and sex-adjusted models, the α (within-sample) and β (between-sample) diversities of taxonomic composition were strongly related to BP indexes ($P<0.001$ for most). In multivariable-adjusted models, β diversity was only associated with diastolic BP ($P=0.032$). However, we observed significant, mainly positive, associations between BP indexes and 45 microbial genera ($P<0.05$), of which 27 belong to the phylum *Firmicutes*. Interestingly, we found mostly negative associations between 19 distinct *Lactobacillus* species and BP indexes ($P<0.05$). Of these, greater abundance of the known probiotic *Lactobacillus paracasei* was associated with lower mean arterial pressure and lower dietary sodium intake ($P<0.001$ for both).

CONCLUSIONS: Although the associations between overall gut taxonomic composition and BP are weak, individuals with hypertension demonstrate changes in several genera. We demonstrate strong negative associations of certain *Lactobacillus* species with sodium intake and BP, highlighting the need for experimental studies.

Key Words: blood pressure ■ gastrointestinal microbiota ■ hypertension ■ *Lactobacillus* ■ salt intake

Dysbiosis of the gut microbiota has been recently linked to various chronic diseases, such as obesity, metabolic syndrome, diabetes mellitus,[1] and cardiovascular disease,[2] and changes in lifestyle.[3] Additional evidence, primarily from animal studies, suggests an association between microbiota and hypertension.[4–7] Furthermore, high salt intake, a risk factor for both hypertension and cardiovascular disease, was shown to deplete certain *Lactobacillus* species in mice while treating the mice with *Lactobacillus* prevented salt-sensitive hypertension.[8] Findings from these studies are consistent with those from human studies, in which consumption of salted snacks has been indicated as a significant

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CLINICAL PERSPECTIVE

What Is New?

- We advance the prior scarce and conflicting knowledge on the associations between gastrointestinal microbes and hypertension in a large, representative population sample using standardized blood pressure measurements, adjustment for relevant confounders, and stool metagenomic sequencing.
- Although the associations between overall gut taxonomic composition and blood pressure are weak, individuals with hypertension demonstrate changes in several microbiota genera, with most of these genera belonging to the *Firmicutes* phylum.
- Interestingly, we also demonstrate strong negative associations of certain *Lactobacillus* species with both dietary sodium intake and blood pressure.

What Are the Clinical Implications?

- The observed associations between the gut microbial composition and hypertension offer novel insights on the potential mechanisms through which diet affects the gut microbiome and blood pressure.

Nonstandard Abbreviations and Acronyms

BP	blood pressure
CARDIA	Coronary Artery Risk Development in Young Adults
ICD-8	*International Classification of Diseases, Eighth Revision*
ICD-9	*International Classification of Diseases, Ninth Revision*
ICD-10	*International Classification of Diseases, Tenth Revision*
KO	Kyoto Encyclopedia of Genes and Genomes Orthology group

correlate of the human gut microbiota.[9] In addition, the gut microbiota has been functionally linked to complications of hypertension, such as arterial thrombosis.[10–12] These prior results therefore highlight the potential of the gut microbiota as a therapeutic target for hypertension.

Despite these promising results from animal studies, human data are scarce and conflicting. In the TwinsUK cohort,[13] self-reported hypertension was not related to 68 various microbiota markers. In another publication based on a subsample of 529 CARDIA (Coronary Artery Risk Development in Young Adults) study participants,[14] an SD increase in gut microbiota α (within-sample) diversity was related to a modest 1.29 mm Hg lower systolic blood pressure (BP; P=0.049). In a commentary, Jama and coauthors[15] proposed several improvements in the experimental design of future studies examining the relation between the microbiota and hypertension, such as the use of standardized BP measurements, adjustment for medication use, and replacing 16S rRNA profiling with metagenome sequencing.

Herein, we aim to advance the current knowledge on the association between the gut metagenome and BP in a well-phenotyped, large random population sample of 6953 individuals while adjusting for relevant confounders, including antihypertensive medication classes. A 24-hour urine sodium sample was available for 829 individuals. We therefore also performed a more focused analysis on the interrelations between sodium intake, gut *Lactobacillus* abundance, and BP to gain additional insight on the potential mechanisms through which the microbiota might affect BP.[8]

METHODS

Availability of Data and Materials

The data that support the findings of this study are available from Finnish Institute for Health and Welfare Biobank (https://thl.fi/en/web/thl-biobank). The data are not publicly available because they contain information that could compromise research participant privacy/consent. The source code for the analyses is openly available at 10.5281/zenodo.3622730.

Study Sample

The Finnish Institute for Health and Welfare has performed population surveys every 5 years since 1972 to monitor the development of cardiovascular risk factors in the Finnish population.[16] A random population sample of 13 437 individuals, aged 25 to 74 years, from 6 geographic regions was invited to take part in the FINRISK 2002 study.[17] Of the 8799 individuals who took part in the FINRISK 2002 study (participation rate, 65.5%), we excluded 1568 who did not provide stool samples, 20 because of low total read count (N<50 000), and 258 because of missing relevant covariates, for a final study sample of 6953 individuals who were included in the analysis. For a subsample of 829 participants, 24-hour urine collection for estimating dietary sodium intake was performed.[18] The study was approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa University Hospital District, and all participants gave written informed consent.

Health Examination and 24-Hour Urine Collection

After completing a questionnaire on sociodemographic information, lifestyles, medications, and medical history at home, the participants attended a physical examination at a local study site. The participants underwent measurements for height and weight. A nurse measured sitting BP 2 times from the right arm using a mercury manometer and a 14×40-cm cuff after a 5-minute rest. Participants in the 24-hour urinary sodium subsample were instructed to start the collection on a Sunday morning and return the container the following day. A sample of urine was frozen at –20°C and later analyzed using an ion-selective electrode (Optima analyzer; Thermo Electron Oy, Vantaa, Finland).[18] Urine sodium excretion was calculated as the product of urine sodium concentration and daily excreted urinary volume.

Stool Sampling and Storage

Stool samples were collected at home after the physical examination in 50-mL Falcon tubes and were mailed to Finnish Institute for Health and Welfare using prepaid packages over 1 to 2 days. The samples were then frozen in –20°C and kept unthawed until 2017, when they underwent metagenomic sequencing.

Stool DNA Extraction and Library Preparation

Microbiota analysis was performed at the University of California, San Diego, using whole genome untargeted shallow shotgun metagenomic sequencing against mapped reference databases, following a previously published protocol.[19] In brief, Illumina-compatible libraries were prepared from isolated DNA and normalized to 5-ng input per sample. Samples were pooled using the iTru[20] dual-indexing system and sequenced using Illumina Hi-Seq 4000 for paired-end 150-bp reads. Sequence reads were mapped against taxonomy using SHOGUN v1.0.5 against National Center for Biotechnology Information RefSeq database (version 82; May 8, 2017).[21,22] Functional profiles were calculated from a combination of observed and predicted Kyoto Encyclopedia of Genes and Genomes Orthology group (KO) annotations from the RefSeq genomes following the predicted parameters of the SHOGUN tool.[21]

Outcome Variables and Covariates

The mean of the 2 measurements was used to determine systolic and diastolic BP. Hypertension was defined as systolic BP ≥140 mm Hg, diastolic BP ≥90 mm Hg, or use of antihypertensive medication. Pulse pressure was defined as systolic minus diastolic BP. Mean arterial pressure was defined as follows: [(2×diastolic BP)+systolic BP]/3.

Body mass index was defined as weight (kilograms) divided by the square of the body height (meters). Participants were asked to assess their leisure-time physical activity by a 4-option multiple choice question. The 4 options for leisure-time activity were (1) sedentary, (2) light activity for >4 hours per week, (3) fitness training or other strenuous exercise for >3 hours per week, and (4) competitive sports. Information on medication use was retrieved from the Finnish national Drug Purchase Register,[23] which captures all prescription drug purchases in Finland. Finnish pharmacies fill prescriptions for a maximum of 3 months, and antihypertensive medication use was defined as a drug purchase occurring within the 4 months preceding baseline. Medications with the following Anatomical Therapeutic Chemical classification[24] codes were considered antihypertensive medications: diuretics (C03*), β blockers (C07*), calcium channel blockers (C08*), and renin–angiotensin system inhibitors (C09*). Prevalent diabetes mellitus was defined as self-reported diabetes mellitus, a previous diagnostic code indicating diabetes mellitus in the nationwide Care Register for Health Care (*International Classification of Diseases, Tenth Revision* [*ICD-10*], codes E10-E14 or *International Classification of Diseases, Eighth Revision/International Classification of Diseases, Ninth Revision* [*ICD-8/ICD-9*], code 250), a previous diabetes mellitus medication purchase (Anatomical Therapeutic Chemical classification code A10*), or special reimbursement code for diabetes mellitus medications in the Drug Reimbursement Register. Smoking was defined by self-reported current daily smoking.

Statistical Analysis

We compared characteristics between individuals who were and were not included in the urinary sodium subsample using the 2-sample T test and the χ^2 test of equality of means. Unless otherwise noted, we adjusted the analyses for age, sex, body mass index, smoking, exercise, diabetes mellitus, diuretic use, β-blocker use, calcium channel blocker use, and renin-angiotensin system inhibitor. We calculated α diversity (Shannon index) using species-level data with the R package *microbiome*.[25] We calculated the dissimilarity matrix (β diversity) and principal coordinates using Bray-Curtis dissimilarity on compositional microbial species-level abundance using R packages *Phyloseq*[26] and *Vegan*.[27] The α diversity is a measure of within-sample diversity (eg, number of microbial species observed or number of questions needed on average to identify random microbe within sample), and the β diversity is a measure of between-sample

diversity (eg, euclidean distance, where each axis represents single species or ratio of number of species shared and number of species in total). We analyzed the associations between β diversity and BP variables using permutational multivariate ANOVA with 999 permutations. We studied common microbial genera (prevalent in at least 1% of sample population with a relative abundance >0.1%) using *DESeq2* with the Benjamini-Hochberg correction.[28,29] We analyzed associations of the genera with (1) BP indexes and (2) 24-hour urinary sodium excretion. We further studied the association between *Lactobacillus* and BP in subgroups by sex and antihypertensive medication. We performed more focused analysis for the detected *Lactobacillus* species, replicating the previous 2 steps. We log(x+1) transformed the KO groups. We used fully adjusted linear regression models to estimate the associations between KO groups and systolic BP. We visualized separately the associations between KO groups and systolic BP using the FuncTree package.[30] For the module, pathway, and biological process layers, we used node sizes that corresponded to the average inverse *P* value of all KO groups that could be assigned to that node. The source code for the analyses is openly available at 10.5281/zenodo.3622730.[31] We used R[32] version 3.6.0 for all statistical analyses.

RESULTS

The characteristics of the main study sample and the 24-hour urinary sodium subsample are reported in Table. A small, but statistically significant, difference between the 2 samples was observed for age (*P*<0.001), diastolic BP (*P*=0.001), pulse pressure (*P*<0.001), heavy exercise (*P*=0.011), β-blocker use (*P*=0.046), and renin-angiotensin system blocker use (*P*=0.030). We observed 134 *Lactobacillus* species (Table S1) and 91 common microbial genera (4.7% of all available genera; Table S2).

In models adjusted for age and sex (Figure 1, Table S3), an SD increase in microbiota α diversity was inversely associated with systolic BP (effect size, −0.54 mm Hg; 95% CI, −0.96 to −0.12 mm Hg; *P*=0.012), diastolic BP (effect size, −0.31 mm Hg; 95% CI, −0.56 to −0.06 mm Hg; *P*=0.016), mean arterial pressure (effect size, −0.39 mm Hg; 95% CI, −0.66 to −0.12 mm Hg; *P*=0.005), and hypertension (odds ratio, 0.91; 95% CI, 0.86–0.96; *P*<0.001). However, α diversity was not related to any BP variable in the multivariable-adjusted models (Figure 1, Table S3).

In the age- and sex-adjusted models, all BP indexes were significantly associated with β diversity (*P*≤0.038 for all; Figure 1, Table S4). The coefficients of determination between β diversity and

Table. Characteristics of the Study Sample

Characteristics	All	Urinary Sodium Subsample	*P* Value
No.	6953	829	
Age, mean (SD), y	49.2 (12.88)	47.2 (10.94)	<0.001
Women, N (%)	3819 (54.9)	460 (55.5)	0.720
BMI, mean (SD), kg/m^2	27.0 (4.66)	26.7 (4.54)	0.117
Systolic BP, mean (SD), mm Hg	135.6 (20.20)	134.6 (18.65)	0.113
Diastolic BP, mean (SD), mm Hg	79.1 (11.23)	80.3 (11.12)	0.001
Pulse pressure, mean (SD), mm Hg	56.5 (16.39)	54.4 (14.16)	<0.001
Arterial pressure, mean (SD), mm Hg	97.9 (12.67)	98.4 (12.40)	0.263
Hypertension, N (%)	3291 (47.3)	378 (45.6)	0.283
Current smoker, N (%)	1637 (23.5)	210 (25.3)	0.189
Diabetes mellitus, N (%)	390 (5.6)	36 (4.3)	0.081
Exercise, N (%)			
Light	1466 (21.1)	167 (20.1)	0.545
Moderate	3922 (56.4)	445 (53.7)	0.109
Heavy	1565 (22.5)	217 (26.2)	0.011
Antihypertensive medication, N (%)	1253 (18.0)	122 (14.7)	
Diuretics	232 (3.3)	20 (2.4)	0.145
β Blockers	714 (10.3)	68 (8.2)	0.046
Calcium channel blockers	293 (4.2)	32 (3.9)	0.670
RAS blockers	569 (8.2)	51 (6.2)	0.030

Continuous variables are presented as mean (SD), and categorical values are presented as count (percentage). Characteristics between individuals who were and were not included in the urinary sodium subsample were compared using the 2-sample T test and the χ^2 test of equality of means. BP indicates blood pressure; BMI, body mass index; and RAS, renin-angiotensin system.

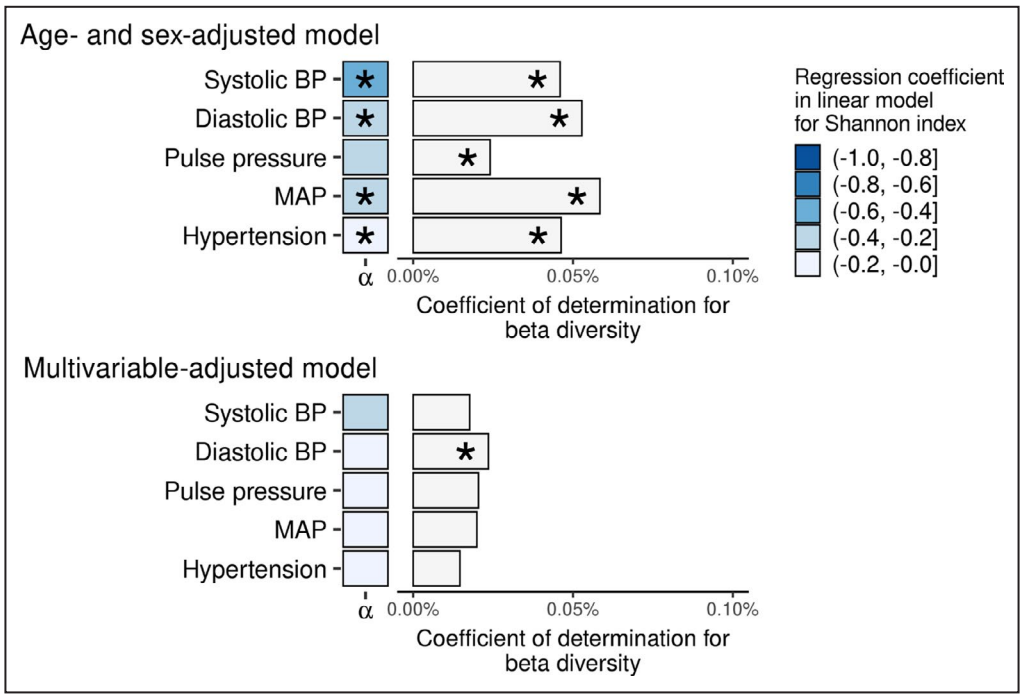

Figure 1. Associations between blood pressure (BP) variables and microbial diversity.
The blue heat maps on the left express the change in BP variables per 1-SD increase in α diversity (Shannon index); log odds are reported for hypertension. The bar plots on the right represent the proportion of variability (R^2) in β diversity (Bray-Curtis distance) explained by BP indexes. Multivariable-adjusted model is adjusted for age, sex, body mass index, smoking, exercise, diuretics, β blockers, calcium channel blockers, and renin-angiotensin system blockers. Significant results are marked with asterisk (P<0.05). α indicates effect size for α diversity; and MAP, mean arterial pressure.

BP variables varied between 0.02% and 0.06%. In multivariable-adjusted models, only diastolic BP (R^2=0.02%; P=0.032) was significantly related to β diversity (Figure 1, Table S4). The first 3 principal coordinate axes explained 31.3% of the variation in bacterial abundances, but visual inspection did not reveal clustering of hypertensive and normotensive individuals (Figure 2).

We then studied the associations between genus-level abundances and BP indexes. We observed 122 significant associations between 45 distinct microbial genera and BP indexes with false discovery

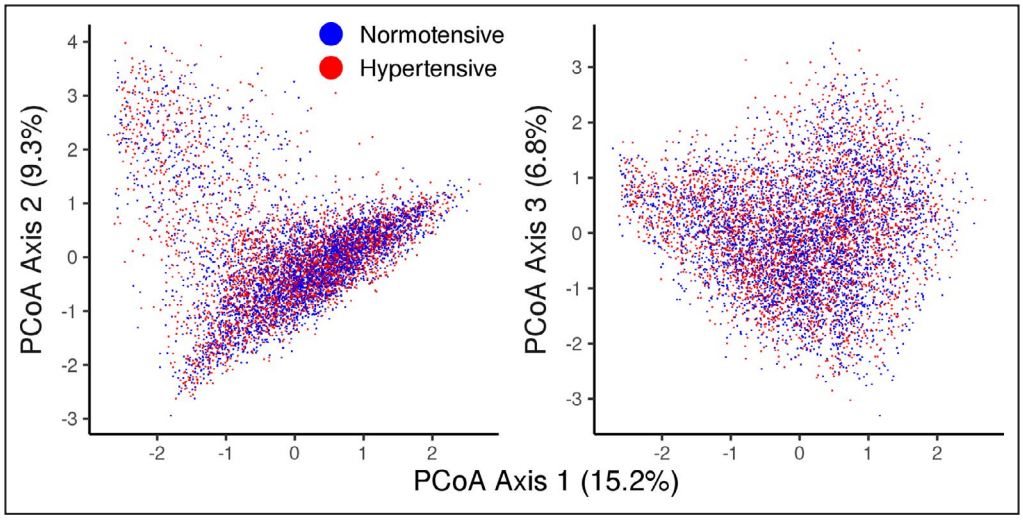

Figure 2. Principal coordinate analysis (PCoA) for species-level bacterial abundances (Bray-Curtis distance).

rate–corrected $P<0.05$ (Figure 3, Table S5). These associations are shown in subgroups by sex and antihypertensive medication use in Figures S1 and S2. The results differed by subgroup for several species. Of all covariates, inclusion of body mass index in the age- and sex-adjusted model resulted in most of the reduction (from 39 to 23; 59%) in the number of significant genera-hypertension associations. The association between the *Lactobacillus* genus and BP indexes was nonsignificant, but species-level analyses revealed 41 significant associations for 19 (14.0%) distinct *Lactobacillus* species with false discovery rate–corrected $P<0.05$. Of these associations, 12 were positive and 29 were negative (Figure 3, Table S6). We observed 481 KO groups associated with systolic BP (false discovery rate–corrected $P<0.05$; Table S7). Internal node calculation revealed that several of the most prominent pathways were related to lipid metabolism, gluconeogenesis, and xenobiotic metabolism (Figure S3).

Finally, we performed a more focused analysis on the association between genus- and species-level *Lactobacillus* abundances and sodium intake in the subsample of participants with 24-hour urinary sodium excretion (mean, 142.3±62.9 mmol) data available. *Lactobacillus* prevalence was 15.5% at the detection limit of 0.1% relative abundance in this subsample. *Lactobacillus* genus was not associated with 24-hour urinary sodium excretion (false discovery rate–corrected $P=0.984$; Figure 4). At the species level (Figure 4), *Lactobacillus paracasei* demonstrated

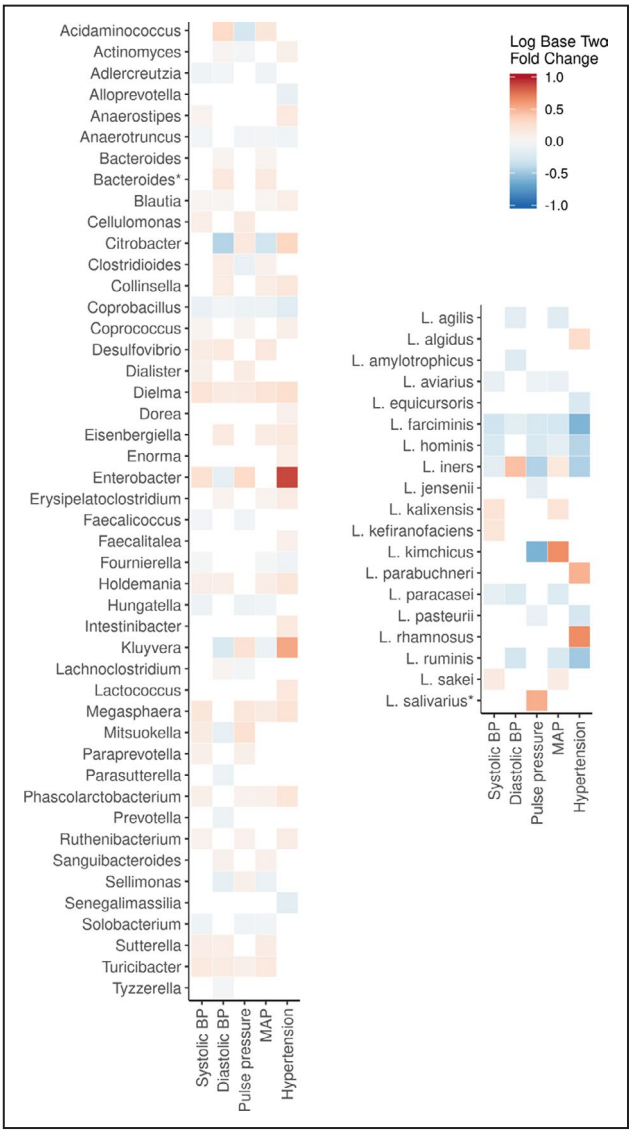

Figure 3. Associations for common microbial genera and *Lactobacillus* species with blood pressure (BP) indexes.
We observed 45 distinct microbial genera and 19 *Lactobacillus* species that were significantly associated with BP indexes using *DESeq2* ($P<0.05$ for all). The heat map expresses the fold change associated with BP indexes in base 2 logarithm ratios of microbial abundances. For hypertension, the range signifies a change of microbial abundance from 0.5 (blue) to 2 (red) times the bacterial abundance in normotensive participants. For continuous variables, the fold change is expressed per 1-SD change in BP variable. The models are adjusted for age, sex, body mass index, smoking, exercise, diuretics, β blockers, calcium channel blockers, and renin-angiotensin system blockers. Association with bacterial plasmid is denoted using asterisk. MAP indicates mean arterial pressure.

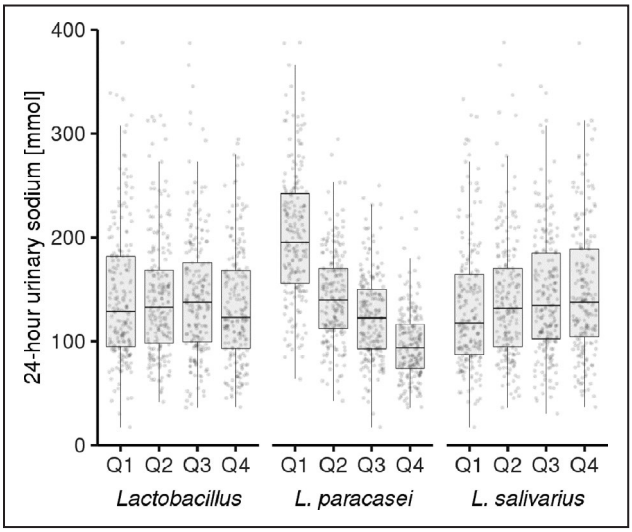

Figure 4. *Lactobacillus* abundance in groups by 24-hour urinary sodium excretion.
We observed significant association between 24-hour urinary sodium excretion and two *Lactobacillus* species with false discovery rate–corrected $P<0.05$ while the genus-level association remained insignificant. For *Lactobacillus paracasei* ($\log_2$ fold change, −0.018±0.002; $P<0.001$), the 24-hour urinary sodium excretion levels were as follows: quartile (Q) 1, 205.4±68.5 mmol; Q2, 143.0±43.8 mmol; Q3, 123.9±41.1 mmol; and Q4, 96.6±33.7 mmol. For *Lactobacillus salivarius* ($\log_2$ fold change, 0.007±0.002; $P=0.004$), the 24-hour urinary sodium excretion levels were as follows: Q1, 132.7±60.3 mmol; Q2, 142.3±63.3 mmol; Q3, 144.0±58.3 mmol; and Q4, 150.1±68.6 mmol. We visualize the associations using quartiles of *DESeq2*-fitted abundances against 24-hour urinary sodium.

a strong, negative association with (log_2 fold change, −0.018±0.002; P<0.001) urinary sodium excretion. *Lactobacillus salivarius* was positively (log_2 fold change, 0.007±0.002; P=0.004) associated with urinary sodium excretion.

DISCUSSION

We investigated the relation between the gut metagenome and objectively measured BP in a large, representative population cohort while adjusting for relevant confounding factors. In age- and sex-adjusted models, we observed strong associations between overall gut taxonomic composition and BP. However, these associations were weaker in multivariable-adjusted models. We observed significant associations between 45 distinct microbial genera and BP indexes, of which 27 belong to the phylum *Firmicutes*. Interestingly, certain *Lactobacillus* species demonstrated strong associations with both BP indexes and 24-hour urinary sodium excretion. Our functional analysis suggests that microbiome-driven processes associated with lipid metabolism, gluconeogenesis, and xenobiotic metabolism may be associated with BP. However, experimental designs with deep microbiome sequencing are needed for more detailed information on the causal/functional mechanisms and, ultimately, the clinical significance of our findings.

Several prior animal studies have suggested an association between intestinal dysbiosis and hypertension.[4] A lower gut microbiota α diversity was reported among hypertensive cases in a small case-control study with a study sample of 11 rats and 17 human patients.[5] In another experimental study by Adnan et al,[6] 6 normotensive rats were gavaged with microbiota from hypertensive rats, which led to increases in the *Firmicutes/Bacteroides* ratio and systolic BP. Finally, Durgan et al[33] suggested that a causal relationship between gut microbial dysbiosis and obstructive sleep apnea–induced hypertension could exist in groups of 6 to 9 rats allocated to high-fat and normal chow diet. These early animal studies have suggested that gut dysbiosis and hypertension could be causally related, offering a basis for large-scale epidemiological studies in humans.

Two previous cohort studies have assessed the association between 16S rRNA-sequenced gut microbiota and hypertension in humans. Jackson et al[13] studied the link between gut microbiota markers and self-reported common diseases in a sample of 2737 TwinsUK study participants. However, no associations were observed between 68 various microbiota markers and self-declared hypertension after correcting for multiple testing. As previously noted,[15] the prevalence of self-reported hypertension (27.6%) in TwinsUK was lower than expected and, therefore, the lack of objective BP measurements could explain these nonsignificant results. In another study by Sun et al,[14] the authors examined the association between gut microbiota and objectively measured BP in 529 CARDIA study participants. This study reported a negative association between gut microbial α diversity and systolic BP. The authors also observed a single significant genus-level association between systolic BP and *Robinsoniella*, which was not included in common microbial genera (prevalence, ≥1%; abundance, ≥0.1%) used in this study. Our study builds on these earlier efforts through the use of shotgun metagenomic sequencing and a large, representative study sample with detailed drug purchase data, standardized BP measurements, and adequate statistical power. Although the multivariable-adjusted associations between overall gut taxonomic composition and BP observed in our study were small, we demonstrate mainly positive associations between 45 microbial genera and BP indexes. A total of 27 of these 45 genera belong to the phylum *Firmicutes*, highlighting the previously observed potentially harmful links of increased *Firmicutes* with hypertension, obesity, diabetes mellitus, and chronic kidney disease.[5,34]

In a previous publication, Wilck et al[8] studied the effects of high dietary salt intake on gut-immune axis through induction of interleukin-17A–producing T-helper 17 cells, which can also contribute to hypertension. The authors reported that *Lactobacillus murinus* was depleted by high dietary salt in mouse models, whereas *L murinus* treatment prevented salt-sensitive hypertension by modulating T-helper 17 cells. In the same study, markedly increased salt intake also led to reduced *Lactobacillus* species abundance, increased T-helper 17 cell levels, and increased BP in a small sample of 12 men and women. In line with the results by Wilck et al,[8] we observed mainly negative, but also positive, associations between *Lactobacillus* species and BP indexes. Although the *Lactobacillus* genus was not associated with 24-hour urinary sodium excretion, we observed a strong association between an increased *L paracasei* abundance and decreased urinary sodium excretion (P<0.001; Figure 4). A positive association between *L salivarius* and sodium excretion was also observed (P=0.004). The directions of the effects for both *Lactobacillus* species were consistent in models for BP and dietary sodium. However, the *Lactobacillus*-related results were somewhat different in subgroups by sex and antihypertensive medication use. In particular, the association between *L paracasei* and BP was stronger in women. The association between *L paracasei* and BP was observed in users and nonusers of antihypertensive medications.

The underlying mechanisms of these associations require further study, but the probiotic supplementation with *L paracasei* has been previously shown to reduce interleukin-17 levels in acutely ill patients and *Lactobacillus casei* group on the whole to induce potential weight loss.[35,36]

Although our study has several advantages, our results must be interpreted in the context of their limitations. First, although fecal sampling is a noninvasive and feasible method for assessing microbiota, it is only a proxy for the gut microbiota. In particular, the stable microbial niche in mucosal layer of the gastrointestinal tract could account for major physiological effects with minor contribution to stool sampling.[37] Second, the relatively long storage time of the samples (15 years at –20°C) could lead to some deterioration of the samples. However, the taxonomic composition of our samples was similar to what has been previously observed in larger cohort studies.[19] Third, metagenomics is a novel field with reported limitations (eg, those related to sequencing and labeling DNA in stool samples).[38] Fourth, despite 24-hour urine collection being a more accurate method for estimating sodium intake than spot urine samples, it remains a cross-sectional snapshot of dietary habits.[39] Fifth, the hypertensive participants of our study may have received instructions to reduce their sodium intake, leading to weaker or reverse causation.

CONCLUSIONS

This study is by far the largest to examine the association between human gut microbiota and objectively measured BP. Although the associations between overall gut taxonomic composition and BP are weak, individuals with hypertension demonstrate changes in several microbiota genera, with most of these genera belonging to the *Firmicutes* phylum. Interestingly, we also demonstrate strong negative associations of certain *Lactobacillus* species with both dietary sodium intake and BP (Figures 3 and 4). This finding provides additional population-level evidence to those from experimental studies demonstrating that distinct *Lactobacillus* species are depleted by high dietary salt, whereas treatment with these same species might prevent salt-sensitive hypertension.[8] Our research needs to be expanded (1) by estimating the effect of the reported associations for public health, (2) by determining the functional role of gut microbiota by combining taxonomic profiling with simultaneous determination of the gut and plasma metabolome, and (3) by conducting additional studies that directly manipulate *Lactobacillus* species levels in the gut to establish the causal relation between these species and hypertension. The observed associations between the gut microbial composition and hypertension offer novel insights on the potential mechanisms through which diet affects the gut microbiome and BP. In addition, our results raise hypotheses on how the microbiome could be manipulated to improve hypertension control.

ARTICLE INFORMATION

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Author contributions: Drs Salomaa, Knight, Lahti, and Niiranen designed the work; Drs Havulinna, Jain, Salido, Sanders, Brennan, Humphrey, Vartiainen, Laatikainen, Jousilahti, and Salomaa acquired the data; Drs Palmu, Salosensaari, Sanders, Lahti, and Niiranen analyzed the data; and Drs Cheng, Inouye, Jain, Jousilahti, Salomaa, Knight, Lahti, and Niiranen supervised the work. All authors wrote the article and gave final approval of the version to be published.

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Disclosures

Dr Salomaa has received honoraria from Novo Nordisk and Sanofi for consultations. He also has ongoing research collaboration with Bayer Ltd (all unrelated to the present study). The remaining authors have no disclosures to report.

Supplementary Materials

Tables S1–S7
Figures S1–S3

REFERENCES

1. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*. 2012;490:55–60.

2. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung Y-M, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011;472:57–63.
3. O'Keefe SJD, Li JV, Lahti L, Ou J, Carbonero F, Mohammed K, Posma JM, Kinross J, Wahl E, Ruder E, et al. Fat, fibre and cancer risk in African Americans and rural Africans. *Nat Commun*. 2015;6:6342.
4. Richards EM, Pepine CJ, Raizada MK, Kim S. The gut, its microbiome, and hypertension. *Curr Hypertens Rep*. 2017;19:36.
5. Yang T, Santisteban MM, Vermali R, Li E, Ahmari N, Carvajal JM, Zadeh M, Gong M, Qi Y, Zubcevic J, et al. Gut dysbiosis is linked to hypertension. *Hypertension*. 2015;65:1331–1340.
6. Adnan S, Nelson JW, Ajami NJ, Venna VR, Petrosino JF, Bryan RM, Durgan DJ. Alterations in the gut microbiota can elicit hypertension in rats. *Physiol Genomics*. 2016;49:96–104.
7. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, Sitaraman SV, Knight R, Ley RE, Gewirtz AT. Metabolic syndrome and altered gut microbiota in mice lacking toll-like receptor 5. *Science*. 2010;328:228–231.
8. Wilck N, Matus MG, Kearney SM, Olesen SW, Forslund K, Bartolomaeus H, Haase S, Mähler A, Balogh A, Markó L, et al. Salt-responsive gut commensal modulates T H 17 axis and disease. *Nature*. 2017;551:585–589.
9. McDonald D, Hyde E, Debelius JW, Morton JT, Gonzalez A, Ackermann G, Aksenov AA, Behsaz B, Brennan C, Chen Y, et al. American gut: an open platform for citizen science microbiome research. *mSystems*. 2018;3:e00031-18. DOI: 10.1128/mSystems.00031-18.
10. Jäckel S, Kiouptsi K, Lillich M, Hendrikx T, Khandagale A, Kollar B, Hörmann N, Reiss C, Subramaniam S, Wilms E, et al. Gut microbiota regulate hepatic von Willebrand factor synthesis and arterial thrombus formation via Toll-like receptor-2. *Blood*. 2017;130:542–553.
11. Kiouptsi K, Jäckel S, Pontarollo G, Grill A, Kuijpers MJE, Wilms E, Weber C, Sommer F, Nagy M, Neideck C, et al. The microbiota promotes arterial thrombosis in low-density lipoprotein receptor-deficient mice. *mBio*. 2019;10:e02298-19. DOI: 10.1128/mBio.02298-19.
12. Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, Li L, Fu X, Wu Y, Mehrabian M, et al. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. *Cell*. 2016;165:111–124.
13. Jackson MA, Verdi S, Maxan M-E, Shin CM, Zierer J, Bowyer RCE, Martin T, Williams FMK, Menni C, Bell JT, et al. Gut microbiota associations with common diseases and prescription medications in a population-based cohort. *Nat Commun*. 2018;9:1–8.
14. Sun S, Lulla A, Sioda M, Winglee K, Wu MC, Jacobs DR, Shikany JM, Lloyd-Jones DM, Launer LJ, Fodor AA, et al. Gut microbiota composition and blood pressure: the CARDIA study. *Hypertension*. 2019;73:998–1006.
15. Jama H, Kaye DM, Marques FZ. Population-based gut microbiome associations with hypertension. *Circ Res*. 2018;123:1185–1187.
16. Borodulin K, Vartiainen E, Peltonen M, Jousilahti P, Juolevi A, Laatikainen T, Mannisto S, Salomaa V, Sundvall J, Puska P. Forty-year trends in cardiovascular risk factors in Finland. *Eur J Public Health*. 2015;25:539–546.
17. Havulinna AS, Sysi-Aho M, Hilvo M, Kauhanen D, Hurme R, Ekroos K, Salomaa V, Laaksonen R. Circulating ceramides predict cardiovascular outcomes in the population-based FINRISK 2002 cohort. *Arterioscler Thromb Vasc Biol*. 2016;36:2424–2430.
18. Laatikainen T, Pietinen P, Valsta L, Sundvall J, Reinivuo H, Tuomilehto J. Sodium in the Finnish diet: 20-year trends in urinary sodium excretion among the adult population. *Eur J Clin Nutr*. 2006;60:965–970.
19. Salosensaari A, Laitinen V, Havulinna AS, Meric G, Cheng S, Perola M, Valsta L, Alfthan G, Inouye M, Watrous JD, et al. Taxonomic signatures of long-term mortality risk in human gut microbiota. *medRxiv*. 2020. DOI: 2019.12.30.19015842
20. Glenn TC, Nilsen RA, Kieran TJ, Sanders JG, Bayona-Vásquez NJ, Finger JW, Pierson TW, Bentley KE, Hoffberg SL, Louha S, et al. Adapterama I: universal stubs and primers for 384 unique dual-indexed or 147,456 combinatorially-indexed Illumina libraries (iTru & iNext). *PeerJ*. 2019;7:e7755.
21. Hillmann B, Al-Ghalith GA, Shields-Cutler RR, Zhu Q, Gohl DM, Beckman KB, Knight R, Knights D. Evaluating the information content of shallow shotgun metagenomics. *mSystems*. 2018;3:e00069-18.
22. O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res*. 2016;44:D733–D745.
23. Statistics on reimbursements for prescription medicines. The Social Insurance Institution of Finland (Kela). https://www.kela.fi/web/en/492. Accessed August 5, 2019.
24. ATC Structure and Principles. WHO Collaborating Centre for Drug Statistics Methodology. https://www.whocc.no/atc/structure_and_principles/. Accessed August 5, 2019.
25. Lahti L, Shetty S. Tools for microbiome analysis in R: version 1.6.0. 2017. Available at: http://microbiome.github.com/microbiome. Accessed May 2, 2019.
26. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*. 2013;8:e61217.
27. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, et al. The Vegan package: version 2.5.6. 2007. https://CRAN.R-project.org/package=vegan.Accessed April 24, 2019.
28. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15:550.
29. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol*. 1995;57:289–300.
30. Uchiyama T, Irie M, Mori H, Kurokawa K, Yamada T. FuncTree: functional analysis and visualization for large-scale omics data. *PLoS One*. 2015;10:e0126967.
31. Palmu J, Salosensaari A, Lahti L, Niiranen T. RRgutbiota: source code for the manuscript association between gut microbiota and blood pressure in a population cohort of 6953 individuals: version 1.6. Zenodo; 2020. DOI: 10.5281/zenodo.3622730. Accessed June 24, 2020.
32. R Core Team. *R: A Language and Environment for Statistical Computing: Version 3.6.0*. R Foundation for Statistical Computing; 2017. https://www.R-project.org/. Accessed May 2, 2019.
33. Durgan DJ, Ganesh BP, Cope JL, Ajami NJ, Phillips SC, Petrosino JF, Hollister EB, Bryan RM. Role of the gut microbiome in obstructive sleep apnea-induced hypertension. *Hypertension*. 2016;67:469–474.
34. Tang WHW, Kitai T, Hazen SL. Gut microbiota in cardiovascular health and disease. *Circ Res*. 2017;120:1183–1196.
35. Angurana SK, Bansal A, Singhi S, Aggarwal R, Jayashree M, Salaria M, Mangat NK. Evaluation of effect of probiotics on cytokine levels in critically ill children with severe sepsis: a double-blind, placebo-controlled trial. *Crit Care Med*. 2018;46:1656–1664.
36. Hill D, Sugrue I, Tobin C, Hill C, Stanton C, Ross RP. The *Lactobacillus casei* group: history and health related applications. *Front Microbiol*. 2018;9:2107. DOI: 10.3389/fmicb.2018.02107.
37. Claesson MJ, Clooney AG, O'Toole PW. A clinician's guide to microbiome analysis. *Nat Rev Gastroenterol Hepatol*. 2017;14:585–595.
38. Thomas AM, Segata N. Multiple levels of the unknown in microbiome research. *BMC Biol*. 2019;17:48.
39. Rakova N, Kitada K, Lerchl K, Dahlmann A, Birukov A, Daub S, Kopp C, Pedchenko T, Zhang Y, Beck L, et al. Increased salt consumption induces body water conservation and decreases fluid intake. *J Clin Invest*. 2017;127:1932–1943.

SUPPLEMENTAL MATERIAL

Table S1. *Lactobacillus* species (N=134).

Ace–Dex	Dio–Kim	Kim–Pla	Pob–Xia
L. acetotolerans	*L. diolivorans*	*L. kimchiensis*	*L. pobuzihii*
L. acidifarinae	*L. equi*	*L. koreensis*	*L. pontis*
L. acidipiscis	*L. equicursoris*	*L. kullabergensis*	*L. psittaci*
L. acidophilus	*L. equigenerosi*	*L. kunkeei*	*L. rapi*
L. agilis	*L. fabifermentans*	*L. lindneri*	*L. rennini*
L. algidus	*L. farciminis*	*L. lindneri**	*L. reuteri*
L. alimentarius	*L. farraginis*	*L. malefermentans*	*L. rhamnosus*
L. amylolyticus	*L. fermentum*	*L. manihotivorans*	*L. rossiae*
L. amylotrophicus	*L. floricola*	*L. mellifer*	*L. ruminis*
L. amylovorus	*L. florum*	*L. mellifer**	*L. saerimneri*
*L. amylovorus**	*L. fructivorans*	*L. mellis*	*L. sakei*
L. animalis	*L. frumenti*	*L. mindensis*	*L. salivarius*
L. antri	*L. fuchuensis*	*L. mucosae*	*L. salivarius**
L. apinorum	*L. gasseri*	*L. mucosae**	*L. sanfranciscensis*
L. apis	*L. gastricus*	*L. nagelii*	*L. sanfranciscensis**
*L. apis**	*L. ghanensis*	*L. nasuensis*	*L. saniviri*
L. aquaticus	*L. gigeriorum*	*L. nodensis*	*L. satsumensis*
L. aviarius	*L. ginsenosidimutans*	*L. odoratitofui*	*L. secaliphilus*
L. bifermentans	*L. graminis*	*L. oeni*	*L. selangorensis*
L. brantae	*L. hamsteri*	*L. oligofermentans*	*L. senioris*
L. brevis	*L. harbinensis*	*L. oryzae*	*L. sharpeae*
*L. brevis**	*L. hayakitensis*	*L. ozensis*	*L. shenzhenensis*
L. buchneri	*L. heilongjiangensis*	*L. panis*	*L. siliginis*
*L. buchneri**	*L. helveticus*	*L. pantheris*	*L. similis*
L. cacaonum	*L. herbarum*	*L. parabrevis*	*L. spicheri*
L. camelliae	*L. hilgardii*	*L. parabuchneri*	*L. sucicola*
L. capillatus	*L. hokkaidonensis*	*L. parabuchneri**	*L. suebicus*
L. ceti	*L. hokkaidonensis**	*L. paracasei*	*L. thailandensis*
L. coleohominis	*L. hominis*	*L. paracasei**	*L. tucceti*
L. composti	*L. hordei*	*L. paracollinoides*	*L. uvarum*
L. concavus	*L. iners*	*L. paracollinoides**	*L. vaccinostercus*
L. coryniformis	*L. ingluviei*	*L. parafarraginis*	*L. vaginalis*
L. crispatus	*L. intestinalis*	*L. paralimentarius*	*L. versmoldensis*
L. crustorum	*L. jensenii*	*L. pasteurii*	*L. vini*
*L. crustorum**	*L. kalixensis*	*L. paucivorans*	*L. wasatchensis*
L. curieae	*L. kefiranofaciens*	*L. perolens*	*L. xiangfangensis*
L. delbrueckii	*L. kefiranofaciens**	*L. plantarum*	
L. dextrinicus	*L. kimchicus*	*L. plantarum**	

Bacterial plasmids are denoted with an asterisk.

Table S2. Common microbial genera (N=91, prevalence ≥1%, abundance ≥0.1%).

Ace–Dia	Die–Lac	Mar–Vei
Acetivibrio	*Dielma*	*Marvinbryantia*
Acidaminococcus	*Dorea*	*Megamonas*
Actinomyces	*Eggerthella*	*Megasphaera*
Adlercreutzia	*Eisenbergiella*	*Methanobrevibacter*
Akkermansia	*Enorma*	*Methanomassiliicoccus*
Alistipes	*Enterobacter*	*Mitsuokella*
Alloprevotella	*Enterococcus*	*Odoribacter*
Anaerostipes	*Erysipelatoclostridium*	*Oscillibacter*
Anaerotruncus	*Escherichia*	*Parabacteroides*
Bacteroides	*Escherichia**	*Paraprevotella*
*Bacteroides**	*Eubacterium*	*Parasutterella*
Barnesiella	*Eubacterium**	*Phascolarctobacterium*
Bifidobacterium	*Faecalibacterium*	*Phocea*
Bilophila	*Faecalicatena*	*Porphyromonas*
Bittarella	*Faecalicoccus*	*Prevotella*
Blautia	*Faecalitalea*	*Roseburia*
Butyricicoccus	*Fournierella*	*Ruminiclostridium*
Butyricimonas	*Gordonibacter*	*Ruminococcus*
Butyrivibrio	*Haemophilus*	*Ruthenibacterium*
Catenibacterium	*Halapricum*	*Sanguibacteroides*
Caudovirales	*Holdemanella*	*Sellimonas*
Cellulomonas	*Holdemania*	*Senegalimassilia*
Citrobacter	*Hungatella*	*Shigella*
Clostridioides	*Intestinibacter*	*Solobacterium*
Clostridium	*Johnsonella*	*Streptococcus*
Collinsella	*Klebsiella*	*Subdoligranulum*
Coprobacillus	*Klebsiella**	*Sutterella*
Coprobacter	*Kluyvera*	*Tannerella*
Coprococcus	*Lachnoanaerobaculum*	*Turicibacter*
Dakarella	*Lachnoclostridium*	*Tyzzerella*
Desulfovibrio	*Lactobacillus*	*Veillonella*
Dialister	*Lactococcus*	

Bacterial plasmids are denoted with an asterisk.

Table S3. Associations between blood pressure indices and microbial alpha diversity.

	Age- and sex-adjusted model		Multivariable-adjusted model	
	β (95%-CI)	p	β (95%-CI)	p
Systolic BP	-0.54 (-0.96 to -0.12)	0.012	-0.20 (-0.62 to 0.21)	0.334
Diastolic BP	-0.31 (-0.56 to -0.06)	0.016	-0.10 (-0.35 to 0.14)	0.404
Mean arterial pressure	-0.39 (-0.66 to -0.12)	0.005	-0.14 (-0.40 to 0.12)	0.304
Pulse pressure	-0.23 (-0.59 to 0.13)	0.203	-0.10 (-0.46 to 0.26)	0.580
Hypertension	0.91 (0.86 to 0.96)	<0.001	0.98 (0.92 to 1.04)	0.498

Multivariable-adjusted model is adjusted for age, sex, BMI, smoking, exercise, diuretics, beta blockers, calcium channel blockers, and renin–angiotensin system blockers. BP, blood pressure.

Table S4. Associations between blood pressure indices and microbial beta diversity.

	Age- and sex adjusted model		Multivariable adjusted model	
	R^2	p	R^2	p
Systolic BP	0.046%	0.001	0.018%	0.195
Diastolic BP	0.053%	0.001	0.024%	0.032
Mean arterial pressure	0.058%	0.001	0.020%	0.126
Pulse pressure	0.024%	0.038	0.020%	0.086
Hypertension	0.046%	0.002	0.015%	0.376

Analysis of variance for beta diversity was calculated using 999 permutations. Multivariable-adjusted model is adjusted for age, sex, BMI, smoking, exercise, diuretics, beta blockers, calcium channel blockers, and renin–angiotensin system blockers. BP, blood pressure.

Table S5. Associations between gut microbial genera and blood pressure indices.

	Systolic BP		Diastolic BP		Pulse pressure		Mean arterial pressure		Hypertension	
	Log2FC±SE	p	Log2FC±SE	p	Log2FC±SE	p	Log2FC±SE	p	Log2FC±SE	p
Acidaminococcus			0.32±0.04	<0.001	-0.29±0.04	<0.001	0.20±0.04	<0.001		
Actinomyces			0.04±0.01	0.011	-0.03±0.01	0.045			0.10±0.03	0.008
Adlercreutzia	-0.07±0.02	0.008	-0.06±0.02	0.042			-0.07±0.02	0.006		
Alloprevotella									-0.12±0.04	0.006
Anaerostipes	0.06±0.02	0.015							0.16±0.04	<0.001
Anaerotruncus	-0.05±0.01	<0.001			-0.05±0.01	<0.001	-0.03±0.01	0.010	-0.06±0.02	0.037
Bacteroides			0.05±0.02	0.027			0.05±0.02	0.022		
*Bacteroides**			0.17±0.04	<0.001			0.16±0.04	<0.001		
Blautia	0.04±0.01	0.015	0.03±0.01	0.038			0.04±0.01	0.008	0.11±0.03	<0.001
Cellulomonas	0.10±0.03	0.016			0.15±0.03	<0.001				
Citrobacter			-0.43±0.04	<0.001	0.17±0.04	<0.001	-0.33±0.04	<0.001	0.35±0.10	0.002
Clostridioides			0.13±0.02	<0.001	-0.12±0.02	<0.001	0.09±0.02	<0.001		
Collinsella			0.13±0.02	<0.001			0.12±0.02	<0.001	0.19±0.05	0.002
Coprobacillus	-0.12±0.02	<0.001	-0.06±0.02	0.006	-0.10±0.02	<0.001	-0.10±0.02	<0.001	-0.20±0.04	<0.001
Coprococcus	0.05±0.01	0.004			0.05±0.01	0.004			0.10±0.03	0.012
Desulfovibrio	0.13±0.03	<0.001	0.16±0.03	<0.001			0.17±0.03	<0.001		
Dialister	0.10±0.04	0.039			0.13±0.04	0.002				
Dielma	0.23±0.03	<0.001	0.14±0.03	<0.001	0.15±0.03	<0.001	0.22±0.03	<0.001	0.28±0.07	0.001
Dorea									0.08±0.03	0.030
Eisenbergiella			0.16±0.03	<0.001			0.13±0.03	<0.001	0.17±0.06	0.018
Enorma									0.11±0.04	0.050
Enterobacter	0.25±0.04	<0.001	-0.15±0.04	<0.001	0.32±0.04	<0.001			0.89±0.09	<0.001
Erysipelatoclostridium			0.06±0.02	0.006			0.05±0.02	0.031	0.13±0.04	0.011
Faecalicoccus	-0.05±0.02	0.041			-0.06±0.02	0.003				
Faecalitalea									0.08±0.03	0.047

	Systolic BP		Diastolic BP		Pulse pressure		Mean arterial pressure		Hypertension	
	Log2FC±SE	p	Log2FC±SE	p	Log2FC±SE	p	Log2FC±SE	p	Log2FC±SE	p
Fournierella	-0.02±0.01	0.022					-0.03±0.01	0.015	-0.07±0.02	0.002
Holdemania	0.10±0.02	<0.001	0.10±0.02	<0.001			0.12±0.02	<0.001	0.20±0.04	<0.001
Hungatella	-0.09±0.02	<0.001			-0.09±0.02	<0.001	-0.06±0.02	0.006		
Intestinibacter									0.17±0.06	0.030
Kluyvera			-0.26±0.04	<0.001	0.24±0.04	<0.001	-0.11±0.04	0.047	0.55±0.09	<0.001
Lachnoclostridium			0.04±0.01	0.008	-0.04±0.01	0.005				
Lactococcus									0.18±0.07	0.047
Megasphaera	0.19±0.03	<0.001			0.19±0.03	<0.001	0.14±0.03	<0.001	0.23±0.07	0.008
Mitsuokella	0.15±0.04	0.001	-0.14±0.04	0.001	0.25±0.04	<0.001				
Paraprevotella	0.09±0.03	0.017			0.10±0.03	0.005				
Parasutterella			-0.10±0.04	0.030						
Phascolarctobacterium	0.10±0.03	0.011			0.08±0.03	0.042	0.08±0.03	0.038	0.22±0.07	0.007
Prevotella			-0.09±0.03	0.048						
Ruthenibacterium	0.07±0.02	0.004			0.08±0.02	0.001			0.12±0.05	0.034
Sanguibacteroides			0.08±0.02	0.005			0.08±0.02	0.006		
Sellimonas			-0.15±0.02	<0.001	0.09±0.02	<0.001	-0.10±0.02	<0.001		
Senegalimassilia									-0.18±0.05	0.003
Solobacterium	-0.08±0.02	0.001			-0.07±0.02	0.005	-0.06±0.02	0.014		
Sutterella	0.11±0.04	0.010	0.11±0.03	0.008			0.13±0.04	0.002		
Turicibacter	0.16±0.04	<0.001	0.13±0.03	<0.001	0.09±0.03	0.030	0.17±0.04	<0.001		
Tyzzerella			-0.04±0.01	0.033						

Models are adjusted for age, sex, BMI, smoking, exercise, diuretics, beta blockers, calcium channel blockers, and renin–angiotensin system blockers. Association with bacterial plasmid is denoted using asterisk. BP, blood pressure. Log2FC, base 2 log of fold change.

Table S6. Associations between *Lactobacillus* species and blood pressure indices.

	Systolic BP		Diastolic BP		Pulse pressure		Mean arterial pressure		Hypertension	
	Log2FC±SE	p	Log2FC±SE	p	Log2FC±SE	p	Log2FC±SE	p	Log2FC±SE	p
L. agilis			-0.19±0.06	0.017			-0.20±0.06	0.013		
L. algidus									0.30±0.09	0.010
L. amylotrophicus			-0.22±0.06	0.003						
L. aviarius	-0.13±0.03	0.001			-0.09±0.03	0.043	-0.12±0.03	0.003		
L. equicursoris									-0.25±0.07	0.003
L. farciminis	-0.33±0.05	<0.001	-0.19±0.04	<0.001	-0.27±0.04	<0.001	-0.30±0.05	<0.001	-0.60±0.10	<0.001
L. hominis	-0.26±0.04	<0.001			-0.27±0.04	<0.001	-0.16±0.04	0.003	-0.42±0.09	<0.001
L. iners	-0.18±0.05	0.013	0.44±0.05	<0.001	-0.43±0.05	<0.001	0.17±0.05	0.02	-0.45±0.11	0.001
L. jensenii					-0.16±0.05	0.011				
L. kalixensis	0.23±0.07	0.013					0.22±0.06	0.013		
L. kefiranofaciens	0.20±0.06	0.025								
L. kimchicus					-0.61±0.19	0.025	0.65±0.20	0.023		
L. parabuchneri									0.50±0.15	0.017
L. paracasei	-0.15±0.04	0.020	-0.23±0.04	<0.001			-0.23±0.04	<0.001		
L. pasteurii					-0.12±0.03	<0.001			-0.29±0.06	<0.001
L. rhamnosus									0.65±0.12	<0.001
L. ruminis			-0.32±0.04	<0.001			-0.26±0.04	<0.001	-0.49±0.08	<0.001
L. sakei	0.15±0.04	0.009					0.13±0.04	0.038		
*L. salivarius**					0.52±0.11	<0.001				

Models are adjusted for age, sex, BMI, smoking, exercise, diuretics, beta blockers, calcium channel blockers, and renin–angiotensin system blockers. Association with bacterial plasmid is denoted using asterisk. BP, blood pressure. Log2FC, base 2 log of fold change.

Table S7. Associations between KO groups and systolic BP.

KO-group	Definition	β (95%-CI)	*P*
K00169	pyruvate ferredoxin oxidoreductase alpha subunit	-0.04 (-0.06 to -0.02)	0.029
K00170	pyruvate ferredoxin oxidoreductase beta subunit	-0.04 (-0.06 to -0.02)	0.029
K00171	pyruvate ferredoxin oxidoreductase delta subunit	-0.04 (-0.06 to -0.02)	0.029
K00172	pyruvate ferredoxin oxidoreductase gamma subunit	-0.04 (-0.06 to -0.02)	0.029
K00402	methyl-coenzyme M reductase gamma subunit	-0.04 (-0.06 to -0.02)	0.029
K00581	tetrahydromethanopterin S-methyltransferase subunit E	-0.04 (-0.06 to -0.02)	0.029
K01854	UDP-galactopyranose mutase	-0.04 (-0.06 to -0.02)	0.029
K02201	pantetheine-phosphate adenylyltransferase	-0.04 (-0.06 to -0.02)	0.029
K02910	large subunit ribosomal protein L31e	-0.04 (-0.06 to -0.02)	0.029
K02944	large subunit ribosomal protein LX	-0.04 (-0.06 to -0.02)	0.029
K03049	DNA-directed RNA polymerase subunit E'	-0.04 (-0.06 to -0.02)	0.029
K03105	signal recognition particle subunit SRP19	-0.04 (-0.06 to -0.02)	0.029
K03136	transcription initiation factor TFIIE subunit alpha	-0.05 (-0.07 to -0.03)	0.029
K03538	ribonuclease P protein subunit POP4	-0.04 (-0.06 to -0.02)	0.029
K04801	replication factor C small subunit	-0.04 (-0.06 to -0.02)	0.029
K07060	endoribonuclease Nob1	-0.04 (-0.06 to -0.02)	0.029
K07133	uncharacterized protein	-0.04 (-0.06 to -0.02)	0.029
K07143	UPF0148 protein	-0.04 (-0.06 to -0.02)	0.029
K07333	archaeal flagellar protein FlaJ	-0.04 (-0.06 to -0.02)	0.029
K07722	CopG family transcriptional regulator, nickel-responsive regulator	-0.04 (-0.06 to -0.02)	0.029
K07730	putative transcriptional regulator	-0.04 (-0.06 to -0.02)	0.029
K07991	archaeal preflagellin peptidase FlaK	-0.04 (-0.06 to -0.02)	0.029
K09713	uncharacterized protein	-0.05 (-0.07 to -0.02)	0.029
K09738	uncharacterized protein	-0.04 (-0.06 to -0.02)	0.029
K12960	5-methylthioadenosine/S-adenosylhomocysteine deaminase	-0.04 (-0.06 to -0.02)	0.029
K13787	geranylgeranyl diphosphate synthase, type I	-0.04 (-0.06 to -0.02)	0.029
K14100	energy-converting hydrogenase A subunit I	-0.04 (-0.06 to -0.02)	0.029
K14104	energy-converting hydrogenase A subunit M	-0.04 (-0.06 to -0.02)	0.029
K14561	U3 small nucleolar ribonucleoprotein protein IMP4	-0.05 (-0.07 to -0.03)	0.029
K14654	2,5-diamino-6-(ribosylamino)-4(3H)-pyrimidinone 5'-phosphate reductase	-0.05 (-0.07 to -0.02)	0.029
K17104	phosphoglycerol geranylgeranyltransferase	-0.04 (-0.06 to -0.02)	0.029
K18237	ribose 1,5-bisphosphate isomerase	-0.04 (-0.06 to -0.02)	0.029
K04800	replication factor C large subunit	-0.04 (-0.06 to -0.02)	0.029
K07739	elongator complex protein 3	-0.04 (-0.06 to -0.02)	0.029
K07142	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine diphosphokinase	-0.04 (-0.06 to -0.02)	0.030
K00180	indolepyruvate ferredoxin oxidoreductase, beta subunit	-0.04 (-0.06 to -0.02)	0.030
K01251	adenosylhomocysteinase	-0.04 (-0.06 to -0.02)	0.030
K02908	large subunit ribosomal protein L30e	-0.04 (-0.06 to -0.02)	0.030
K03234	elongation factor 2	-0.04 (-0.06 to -0.02)	0.030

K06875	programmed cell death protein 5	-0.04 (-0.06 to -0.02)	0.030
K08981	putative membrane protein	-0.04 (-0.06 to -0.02)	0.030
K17884	archaetidylinositol phosphate synthase	-0.04 (-0.06 to -0.02)	0.030
K00651	homoserine O-succinyltransferase/O-acetyltransferase	-0.04 (-0.06 to -0.02)	0.030
K02885	large subunit ribosomal protein L19e	-0.04 (-0.06 to -0.02)	0.030
K04518	prephenate dehydratase	-0.04 (-0.06 to -0.02)	0.030
K06218	mRNA interferase RelE/StbE	-0.04 (-0.06 to -0.02)	0.030
K06898	pyridinium-3,5-biscarboxylic acid mononucleotide synthase	-0.04 (-0.06 to -0.02)	0.030
K07166	ACT domain-containing protein	-0.04 (-0.06 to -0.02)	0.030
K18882	DNA primase large subunit	-0.04 (-0.06 to -0.02)	0.030
K03047	DNA-directed RNA polymerase subunit D	-0.04 (-0.06 to -0.02)	0.032
K16306	fructose-bisphosphate aldolase / 2-amino-3,7-dideoxy-D-threo-hept-6-ulosonate synthase	-0.04 (-0.06 to -0.02)	0.032
K00611	ornithine carbamoyltransferase	-0.04 (-0.06 to -0.02)	0.033
K09157	uncharacterized protein	-0.04 (-0.06 to -0.02)	0.033
K00262	glutamate dehydrogenase (NADP+)	-0.04 (-0.06 to -0.02)	0.034
K00319	methylenetetrahydromethanopterin dehydrogenase	-0.04 (-0.06 to -0.02)	0.034
K01912	phenylacetate-CoA ligase	-0.04 (-0.06 to -0.02)	0.034
K07727	putative transcriptional regulator	-0.04 (-0.06 to -0.02)	0.034
K00058	D-3-phosphoglycerate dehydrogenase / 2-oxoglutarate reductase	-0.04 (-0.06 to -0.02)	0.034
K00177	2-oxoglutarate ferredoxin oxidoreductase subunit gamma	-0.04 (-0.06 to -0.02)	0.034
K00179	indolepyruvate ferredoxin oxidoreductase, alpha subunit	-0.04 (-0.06 to -0.02)	0.034
K00184	dimethyl sulfoxide reductase iron-sulfur subunit	-0.04 (-0.06 to -0.02)	0.034
K00185	dimethyl sulfoxide reductase membrane subunit	-0.04 (-0.06 to -0.02)	0.034
K00582	tetrahydromethanopterin S-methyltransferase subunit F	-0.04 (-0.06 to -0.02)	0.034
K00818	acetylornithine aminotransferase	-0.04 (-0.06 to -0.02)	0.034
K00930	acetylglutamate kinase	-0.04 (-0.06 to -0.02)	0.034
K00940	nucleoside-diphosphate kinase	-0.04 (-0.06 to -0.02)	0.034
K01012	biotin synthase	-0.04 (-0.06 to -0.02)	0.034
K01091	phosphoglycolate phosphatase	-0.04 (-0.06 to -0.02)	0.034
K01151	deoxyribonuclease IV	-0.04 (-0.06 to -0.02)	0.034
K01421	putative membrane protein	-0.04 (-0.06 to -0.02)	0.034
K01439	succinyl-diaminopimelate desuccinylase	-0.04 (-0.06 to -0.02)	0.034
K01478	arginine deiminase	-0.04 (-0.06 to -0.02)	0.034
K01482	dimethylargininase	-0.04 (-0.06 to -0.02)	0.034
K01523	phosphoribosyl-ATP pyrophosphohydrolase	-0.04 (-0.06 to -0.02)	0.034
K01693	imidazoleglycerol-phosphate dehydratase	-0.04 (-0.06 to -0.02)	0.034
K01885	glutamyl-tRNA synthetase	-0.04 (-0.06 to -0.02)	0.034
K02195	heme exporter protein C	-0.04 (-0.06 to -0.02)	0.034
K02198	cytochrome c-type biogenesis protein CcmF	-0.04 (-0.06 to -0.02)	0.034
K02203	phosphoserine / homoserine phosphotransferase	-0.04 (-0.06 to -0.02)	0.034
K02282	pilus assembly protein CpaE	-0.04 (-0.06 to -0.02)	0.034
K02319	DNA polymerase, archaea type	-0.04 (-0.06 to -0.02)	0.034

K02434	aspartyl-tRNA(Asn)/glutamyl-tRNA(Gln) amidotransferase subunit B	-0.04 (-0.06 to -0.02)	0.034
K02573	ferredoxin-type protein NapG	-0.04 (-0.06 to -0.02)	0.034
K02574	ferredoxin-type protein NapH	-0.04 (-0.06 to -0.02)	0.034
K02912	large subunit ribosomal protein L32e	-0.04 (-0.06 to -0.02)	0.034
K02929	large subunit ribosomal protein L44e	-0.04 (-0.06 to -0.02)	0.034
K02930	large subunit ribosomal protein L4e	-0.04 (-0.06 to -0.02)	0.034
K03205	type IV secretion system protein VirD4	-0.04 (-0.06 to -0.02)	0.034
K03385	nitrite reductase (cytochrome c-552)	-0.04 (-0.06 to -0.02)	0.034
K03465	thymidylate synthase (FAD)	-0.04 (-0.06 to -0.02)	0.034
K03498	trk system potassium uptake protein	-0.04 (-0.06 to -0.02)	0.034
K03499	trk system potassium uptake protein	-0.04 (-0.06 to -0.02)	0.034
K03540	ribonuclease P protein subunit RPR2	-0.04 (-0.06 to -0.02)	0.034
K03655	ATP-dependent DNA helicase RecG	-0.04 (-0.06 to -0.02)	0.034
K03758	arginine:ornithine antiporter / lysine permease	-0.04 (-0.06 to -0.02)	0.034
K04041	fructose-1,6-bisphosphatase III	-0.04 (-0.06 to -0.02)	0.034
K04655	hydrogenase expression/formation protein HypE	-0.04 (-0.06 to -0.02)	0.034
K04656	hydrogenase maturation protein HypF	-0.04 (-0.06 to -0.02)	0.034
K05780	alpha-D-ribose 1-methylphosphonate 5-triphosphate synthase subunit PhnL	-0.04 (-0.06 to -0.02)	0.034
K05781	putative phosphonate transport system ATP-binding protein	-0.04 (-0.06 to -0.02)	0.034
K06163	alpha-D-ribose 1-methylphosphonate 5-phosphate C-P lyase	-0.04 (-0.06 to -0.02)	0.034
K06914	tyramine---L-glutamate ligase	-0.04 (-0.06 to -0.02)	0.034
K06915	uncharacterized protein	-0.04 (-0.06 to -0.02)	0.034
K06963	tRNA acetyltransferase TAN1	-0.04 (-0.06 to -0.02)	0.034
K07079	uncharacterized protein	-0.04 (-0.06 to -0.02)	0.034
K07154	serine/threonine-protein kinase HipA	-0.04 (-0.06 to -0.02)	0.034
K07307	anaerobic dimethyl sulfoxide reductase subunit B	-0.04 (-0.06 to -0.02)	0.034
K07308	anaerobic dimethyl sulfoxide reductase subunit C	-0.04 (-0.06 to -0.02)	0.034
K07309	Tat-targeted selenate reductase subunit YnfE	-0.04 (-0.06 to -0.02)	0.034
K07580	Zn-ribbon RNA-binding protein	-0.04 (-0.06 to -0.02)	0.034
K07776	two-component system, OmpR family, response regulator RegX3	-0.04 (-0.06 to -0.02)	0.034
K07979	GntR family transcriptional regulator	-0.04 (-0.06 to -0.02)	0.034
K08372	putative serine protease PepD	-0.04 (-0.06 to -0.02)	0.034
K08600	sortase B	-0.04 (-0.06 to -0.02)	0.034
K08979	putative membrane protein	-0.04 (-0.06 to -0.02)	0.034
K09121	pyridinium-3,5-bisthiocarboxylic acid mononucleotide nickel chelatase	-0.04 (-0.06 to -0.02)	0.034
K10040	aspartate/glutamate/glutamine transport system permease protein	-0.04 (-0.06 to -0.02)	0.034
K10206	LL-diaminopimelate aminotransferase	-0.04 (-0.06 to -0.02)	0.034
K11176	IMP cyclohydrolase	-0.04 (-0.06 to -0.02)	0.034
K11755	phosphoribosyl-AMP cyclohydrolase / phosphoribosyl-ATP pyrophosphohydrolase	-0.04 (-0.06 to -0.02)	0.034
K11913	type VI secretion system protein	-0.04 (-0.06 to -0.02)	0.034

K12257	SecD/SecF fusion protein	-0.04 (-0.06 to -0.02)	0.034
K13252	putrescine carbamoyltransferase	-0.04 (-0.06 to -0.02)	0.034
K13640	MerR family transcriptional regulator, heat shock protein HspR	-0.04 (-0.06 to -0.02)	0.034
K13812	bifunctional enzyme Fae/Hps	-0.04 (-0.06 to -0.02)	0.034
K14088	ech hydrogenase subunit C	-0.04 (-0.06 to -0.02)	0.034
K14188	D-alanine--poly(phosphoribitol) ligase subunit 2	-0.04 (-0.06 to -0.02)	0.034
K15429	tRNA (guanine37-N1)-methyltransferase	-0.04 (-0.06 to -0.02)	0.034
K16214	UDP-N-acetylglucosamine kinase	-0.04 (-0.06 to -0.02)	0.034
K16793	methanogen homoaconitase small subunit	-0.04 (-0.06 to -0.02)	0.034
K20265	glutamate:GABA antiporter	-0.04 (-0.06 to -0.02)	0.034
K00145	N-acetyl-gamma-glutamyl-phosphate reductase	-0.04 (-0.06 to -0.01)	0.034
K00176	2-oxoglutarate ferredoxin oxidoreductase subunit delta	-0.04 (-0.06 to -0.01)	0.034
K00399	methyl-coenzyme M reductase alpha subunit	-0.04 (-0.06 to -0.01)	0.034
K00613	glycine amidinotransferase	-0.04 (-0.06 to -0.01)	0.034
K00666	fatty-acyl-CoA synthase	-0.04 (-0.06 to -0.01)	0.034
K00846	ketohexokinase	-0.04 (-0.06 to -0.01)	0.034
K01372	bleomycin hydrolase	-0.04 (-0.06 to -0.01)	0.034
K01470	creatinine amidohydrolase	-0.04 (-0.06 to -0.01)	0.034
K01661	naphthoate synthase	-0.04 (-0.06 to -0.01)	0.034
K02069	putative ABC transport system permease protein	-0.04 (-0.06 to -0.01)	0.034
K02435	aspartyl-tRNA(Asn)/glutamyl-tRNA(Gln) amidotransferase subunit C	-0.04 (-0.06 to -0.01)	0.034
K03521	electron transfer flavoprotein beta subunit	-0.04 (-0.06 to -0.01)	0.034
K03552	holliday junction resolvase Hjr	-0.04 (-0.06 to -0.01)	0.034
K03739	membrane protein involved in D-alanine export	-0.04 (-0.06 to -0.02)	0.034
K04653	hydrogenase expression/formation protein HypC	-0.04 (-0.06 to -0.01)	0.034
K05516	curved DNA-binding protein	-0.04 (-0.06 to -0.01)	0.034
K06162	alpha-D-ribose 1-methylphosphonate 5-triphosphate diphosphatase	-0.04 (-0.06 to -0.01)	0.034
K07171	mRNA interferase MazF	-0.04 (-0.06 to -0.01)	0.034
K07306	anaerobic dimethyl sulfoxide reductase subunit A	-0.04 (-0.06 to -0.02)	0.034
K07402	xanthine dehydrogenase accessory factor	-0.04 (-0.06 to -0.01)	0.034
K07562	nonsense-mediated mRNA decay protein 3	-0.04 (-0.06 to -0.01)	0.034
K07581	RNA-binding protein	-0.04 (-0.06 to -0.02)	0.034
K08177	MFS transporter, OFA family, oxalate/formate antiporter	-0.04 (-0.06 to -0.02)	0.034
K08999	uncharacterized protein	-0.04 (-0.06 to -0.01)	0.034
K09013	Fe-S cluster assembly ATP-binding protein	-0.04 (-0.06 to -0.01)	0.034
K09167	uncharacterized protein	-0.04 (-0.06 to -0.01)	0.034
K10536	agmatine deiminase	-0.04 (-0.06 to -0.01)	0.034
K11358	aspartate aminotransferase	-0.04 (-0.06 to -0.02)	0.034
K11912	serine/threonine-protein kinase PpkA	-0.04 (-0.06 to -0.01)	0.034
K12136	hydrogenase-4 component A	-0.04 (-0.06 to -0.02)	0.034
K12143	hydrogenase-4 component H	-0.04 (-0.06 to -0.02)	0.034
K18926	MFS transporter, DHA2 family, lincomycin resistance protein	-0.04 (-0.06 to -0.01)	0.034

K08744	cardiolipin synthase (CMP-forming)	-0.04 (-0.06 to -0.01)	0.034
K09790	uncharacterized protein	-0.04 (-0.06 to -0.01)	0.034
K14086	ech hydrogenase subunit A	-0.04 (-0.06 to -0.01)	0.034
K02322	DNA polymerase II large subunit	-0.04 (-0.06 to -0.01)	0.035
K00123	formate dehydrogenase major subunit	-0.03 (-0.06 to -0.01)	0.035
K01895	acetyl-CoA synthetase	-0.03 (-0.06 to -0.01)	0.035
K01959	pyruvate carboxylase subunit A	-0.03 (-0.06 to -0.01)	0.035
K02279	pilus assembly protein CpaB	-0.03 (-0.06 to -0.01)	0.035
K02927	ubiquitin-large subunit ribosomal protein L40e	-0.04 (-0.06 to -0.01)	0.035
K03231	elongation factor 1-alpha	-0.04 (-0.06 to -0.01)	0.035
K03522	electron transfer flavoprotein alpha subunit	-0.03 (-0.06 to -0.01)	0.035
K03783	purine-nucleoside phosphorylase	-0.03 (-0.06 to -0.01)	0.035
K06982	pantoate kinase	-0.03 (-0.06 to -0.01)	0.035
K07558	tRNA nucleotidyltransferase (CCA-adding enzyme)	-0.04 (-0.06 to -0.01)	0.035
K09730	uncharacterized protein	-0.04 (-0.06 to -0.01)	0.035
K11741	quaternary ammonium compound-resistance protein SugE	-0.03 (-0.06 to -0.01)	0.035
K01814	phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	-0.03 (-0.06 to -0.01)	0.035
K00053	ketol-acid reductoisomerase	-0.03 (-0.06 to -0.01)	0.035
K00931	glutamate 5-kinase	-0.03 (-0.06 to -0.01)	0.035
K02433	aspartyl-tRNA(Asn)/glutamyl-tRNA(Gln) amidotransferase subunit A	-0.03 (-0.06 to -0.01)	0.035
K02614	acyl-CoA thioesterase	-0.03 (-0.06 to -0.01)	0.035
K02683	DNA primase small subunit	-0.03 (-0.06 to -0.01)	0.035
K03151	tRNA uracil 4-sulfurtransferase	-0.03 (-0.06 to -0.01)	0.035
K03622	archaea-specific DNA-binding protein	-0.03 (-0.06 to -0.01)	0.035
K06206	sugar fermentation stimulation protein A	-0.03 (-0.06 to -0.01)	0.035
K06921	uncharacterized protein	-0.03 (-0.06 to -0.01)	0.035
K07164	uncharacterized protein	-0.03 (-0.06 to -0.01)	0.035
K07452	5-methylcytosine-specific restriction enzyme B	-0.03 (-0.06 to -0.01)	0.035
K09693	teichoic acid transport system ATP-binding protein	-0.03 (-0.06 to -0.01)	0.035
K02922	large subunit ribosomal protein L37e	-0.03 (-0.06 to -0.01)	0.035
K00052	3-isopropylmalate dehydrogenase	-0.03 (-0.05 to -0.01)	0.035
K00286	pyrroline-5-carboxylate reductase	-0.03 (-0.05 to -0.01)	0.035
K00817	histidinol-phosphate aminotransferase	-0.03 (-0.05 to -0.01)	0.035
K00926	carbamate kinase	-0.03 (-0.05 to -0.01)	0.035
K01129	dGTPase	-0.03 (-0.05 to -0.01)	0.035
K01704	3-isopropylmalate/(R)-2-methylmalate dehydratase small subunit	-0.03 (-0.05 to -0.01)	0.035
K02042	phosphonate transport system permease protein	-0.03 (-0.05 to -0.01)	0.035
K02068	putative ABC transport system ATP-binding protein	-0.03 (-0.05 to -0.01)	0.035
K02653	type IV pilus assembly protein PilC	-0.03 (-0.05 to -0.01)	0.035
K03313	Na+:H+ antiporter, NhaA family	-0.03 (-0.05 to -0.01)	0.035
K03502	DNA polymerase V	-0.03 (-0.05 to -0.01)	0.035
K03688	ubiquinone biosynthesis protein	-0.03 (-0.05 to -0.01)	0.035

K05919	superoxide reductase	-0.03 (-0.05 to -0.01)	0.035
K06164	alpha-D-ribose 1-methylphosphonate 5-triphosphate synthase subunit PhnI	-0.03 (-0.05 to -0.01)	0.035
K06165	alpha-D-ribose 1-methylphosphonate 5-triphosphate synthase subunit PhnH	-0.03 (-0.05 to -0.01)	0.035
K06166	alpha-D-ribose 1-methylphosphonate 5-triphosphate synthase subunit PhnG	-0.03 (-0.06 to -0.01)	0.035
K06196	cytochrome c-type biogenesis protein	-0.03 (-0.05 to -0.01)	0.035
K06972	presequence protease	-0.03 (-0.05 to -0.01)	0.035
K07045	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.035
K07126	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.035
K07405	alpha-amylase	-0.03 (-0.05 to -0.01)	0.035
K08659	dipeptidase	-0.03 (-0.05 to -0.01)	0.035
K09773	[pyruvate, water dikinase]-phosphate phosphotransferase /	-0.03 (-0.06 to -0.01)	0.035
K11928	sodium/proline symporter	-0.03 (-0.05 to -0.01)	0.035
K12410	NAD-dependent deacetylase	-0.03 (-0.05 to -0.01)	0.035
K14564	nucleolar protein 56	-0.03 (-0.06 to -0.01)	0.035
K17828	dihydroorotate dehydrogenase (NAD+) catalytic subunit	-0.03 (-0.05 to -0.01)	0.035
K17870	NADH oxidase (H2O2-forming)	-0.03 (-0.06 to -0.01)	0.035
K03116	sec-independent protein translocase protein TatA	-0.03 (-0.05 to -0.01)	0.036
K07220	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.036
K16153	glycogen phosphorylase/synthase	-0.03 (-0.05 to -0.01)	0.036
K19067	cyclohexane-1-carbonyl-CoA dehydrogenase	-0.03 (-0.05 to -0.01)	0.036
K01733	threonine synthase	-0.03 (-0.05 to -0.01)	0.036
K02654	leader peptidase (prepilin peptidase) / N-methyltransferase	-0.03 (-0.05 to -0.01)	0.036
K03183	demethylmenaquinone methyltransferase / 2-methoxy-6-polyprenyl-1,4-benzoquinol methylase	-0.03 (-0.05 to -0.01)	0.036
K03307	solute:Na+ symporter, SSS family	-0.03 (-0.05 to -0.01)	0.036
K03340	diaminopimelate dehydrogenase	-0.03 (-0.05 to -0.01)	0.036
K06943	nucleolar GTP-binding protein	-0.03 (-0.05 to -0.01)	0.036
K06962	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.036
K07446	tRNA (guanine10-N2)-dimethyltransferase	-0.03 (-0.05 to -0.01)	0.036
K12511	tight adherence protein C	-0.03 (-0.05 to -0.01)	0.036
K16792	methanogen homoaconitase large subunit	-0.03 (-0.05 to -0.01)	0.036
K19955	alcohol dehydrogenase	-0.03 (-0.05 to -0.01)	0.036
K07144	5-(aminomethyl)-3-furanmethanol phosphate kinase	-0.03 (-0.05 to -0.01)	0.036
K01426	amidase	-0.03 (-0.05 to -0.01)	0.037
K00174	2-oxoglutarate/2-oxoacid ferredoxin oxidoreductase subunit alpha	-0.03 (-0.05 to -0.01)	0.037
K00794	6,7-dimethyl-8-ribityllumazine synthase	-0.03 (-0.05 to -0.01)	0.037
K01649	2-isopropylmalate synthase	-0.03 (-0.05 to -0.01)	0.037
K01687	dihydroxy-acid dehydratase	-0.03 (-0.05 to -0.01)	0.037
K02875	large subunit ribosomal protein L14e	-0.03 (-0.05 to -0.01)	0.037
K03316	monovalent cation:H+ antiporter, CPA1 family	-0.03 (-0.05 to -0.01)	0.037
K08963	methylthioribose-1-phosphate isomerase	-0.03 (-0.05 to -0.01)	0.037
K03243	translation initiation factor 5B	-0.03 (-0.05 to -0.01)	0.037

K00147	glutamate-5-semialdehyde dehydrogenase	-0.03 (-0.05 to -0.01)	0.037
K03722	ATP-dependent DNA helicase DinG	-0.03 (-0.05 to -0.01)	0.037
K16926	energy-coupling factor transport system substrate-specific component	-0.03 (-0.05 to -0.01)	0.037
K02197	cytochrome c-type biogenesis protein CcmE	-0.03 (-0.05 to -0.01)	0.037
K03242	translation initiation factor 2 subunit 3	-0.03 (-0.05 to -0.01)	0.037
K08096	GTP cyclohydrolase IIa	-0.03 (-0.05 to -0.01)	0.037
K18828	tRNA(fMet)-specific endonuclease VapC	-0.03 (-0.05 to -0.01)	0.037
K03306	inorganic phosphate transporter, PiT family	-0.03 (-0.05 to -0.01)	0.038
K15449	tRNA wybutosine-synthesizing protein 1	-0.03 (-0.05 to -0.01)	0.038
K08352	thiosulfate reductase / polysulfide reductase chain A	-0.03 (-0.05 to -0.01)	0.038
K10254	oleate hydratase	-0.03 (-0.05 to -0.01)	0.038
K00313	electron transfer flavoprotein-quinone oxidoreductase	-0.03 (-0.05 to -0.01)	0.039
K00442	coenzyme F420 hydrogenase subunit delta	-0.03 (-0.05 to -0.01)	0.039
K00868	pyridoxine kinase	-0.03 (-0.05 to -0.01)	0.039
K01710	dTDP-glucose 4,6-dehydratase	-0.03 (-0.05 to -0.01)	0.039
K02564	glucosamine-6-phosphate deaminase	-0.03 (-0.05 to -0.01)	0.039
K02669	twitching motility protein PilT	-0.03 (-0.05 to -0.01)	0.039
K03639	GTP 3',8-cyclase	-0.03 (-0.05 to -0.01)	0.039
K04751	nitrogen regulatory protein P-II 1	-0.03 (-0.05 to -0.01)	0.039
K09138	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.039
K12589	exosome complex component RRP42	-0.03 (-0.05 to -0.01)	0.039
K15888	tritrans,polycis-undecaprenyl-diphosphate synthase	-0.03 (-0.05 to -0.01)	0.039
K16785	energy-coupling factor transport system permease protein	-0.03 (-0.05 to -0.01)	0.039
K00548	5-methyltetrahydrofolate--homocysteine methyltransferase	-0.03 (-0.05 to -0.01)	0.039
K00764	amidophosphoribosyltransferase	-0.03 (-0.05 to -0.01)	0.039
K01524	exopolyphosphatase / guanosine-5'-triphosphate,3'-diphosphate pyrophosphatase	-0.03 (-0.05 to -0.01)	0.039
K01571	oxaloacetate decarboxylase (Na+ extruding) subunit alpha	-0.03 (-0.05 to -0.01)	0.039
K02019	molybdate transport system regulatory protein	-0.03 (-0.05 to -0.01)	0.039
K02283	pilus assembly protein CpaF	-0.03 (-0.05 to -0.01)	0.039
K03427	type I restriction enzyme M protein	-0.03 (-0.05 to -0.01)	0.039
K03620	Ni/Fe-hydrogenase 1 B-type cytochrome subunit	-0.03 (-0.05 to -0.01)	0.039
K05837	rod shape determining protein RodA	-0.03 (-0.05 to -0.01)	0.039
K06935	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.039
K06953	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.039
K08681	5'-phosphate synthase pdxT subunit	-0.03 (-0.05 to -0.01)	0.039
K11041	exfoliative toxin A/B	-0.03 (-0.05 to -0.01)	0.039
K11927	ATP-dependent RNA helicase RhlE	-0.03 (-0.05 to -0.01)	0.039
K00762	orotate phosphoribosyltransferase	-0.03 (-0.05 to -0.01)	0.039
K01006	pyruvate, orthophosphate dikinase	-0.03 (-0.05 to -0.01)	0.039
K01499	methenyltetrahydromethanopterin cyclohydrolase	-0.03 (-0.05 to -0.01)	0.039
K01653	acetolactate synthase I/III small subunit	-0.03 (-0.05 to -0.01)	0.039
K01681	aconitate hydratase	-0.03 (-0.05 to -0.01)	0.039

K02823	dihydroorotate dehydrogenase electron transfer subunit	-0.03 (-0.05 to -0.01)	0.039
K03051	DNA-directed RNA polymerase subunit F	-0.03 (-0.05 to -0.01)	0.039
K05715	2-phosphoglycerate kinase	-0.03 (-0.05 to -0.01)	0.039
K07533	foldase protein PrsA	-0.03 (-0.05 to -0.01)	0.039
K15830	formate hydrogenlyase subunit 5	-0.03 (-0.05 to -0.01)	0.039
K15669	D-glycero-alpha-D-manno-heptose 1-phosphate guanylyltransferase	-0.03 (-0.05 to -0.01)	0.040
K00789	S-adenosylmethionine synthetase	-0.03 (-0.05 to -0.01)	0.040
K02217	ferritin	-0.03 (-0.05 to -0.01)	0.040
K00555	tRNA (guanine26-N2/guanine27-N2)-dimethyltransferase	-0.03 (-0.05 to -0.01)	0.040
K02017	molybdate transport system ATP-binding protein	-0.03 (-0.05 to -0.01)	0.040
K06610	MFS transporter, SP family, inositol transporter	-0.03 (-0.05 to -0.01)	0.040
K09153	small membrane protein	-0.03 (-0.05 to -0.01)	0.040
K00003	homoserine dehydrogenase	-0.03 (-0.05 to -0.01)	0.041
K00175	2-oxoglutarate/2-oxoacid ferredoxin oxidoreductase subunit beta	-0.03 (-0.05 to -0.01)	0.041
K00848	rhamnulokinase	-0.03 (-0.05 to -0.01)	0.041
K01737	6-pyruvoyltetrahydropterin/6-carboxytetrahydropterin synthase	-0.03 (-0.05 to -0.01)	0.041
K01915	glutamine synthetase	-0.03 (-0.05 to -0.01)	0.041
K01937	CTP synthase	-0.03 (-0.05 to -0.01)	0.041
K02977	ubiquitin-small subunit ribosomal protein S27Ae	-0.03 (-0.05 to -0.01)	0.041
K03106	signal recognition particle subunit SRP54	-0.03 (-0.05 to -0.01)	0.041
K03150	2-iminoacetate synthase	-0.03 (-0.05 to -0.01)	0.041
K03696	ATP-dependent Clp protease ATP-binding subunit ClpC	-0.03 (-0.05 to -0.01)	0.041
K04773	protease IV	-0.03 (-0.05 to -0.01)	0.041
K07569	RNA-binding protein	-0.03 (-0.05 to -0.01)	0.041
K07704	two-component system, LytTR family, sensor histidine kinase LytS	-0.03 (-0.05 to -0.01)	0.041
K10117	raffinose/stachyose/melibiose transport system substrate-binding protein	-0.03 (-0.05 to -0.01)	0.041
K14652	3,4-dihydroxy 2-butanone 4-phosphate synthase / GTP cyclohydrolase II	-0.03 (-0.05 to -0.01)	0.041
K18350	two-component system, OmpR family, sensor histidine kinase VanS	-0.03 (-0.05 to -0.01)	0.041
K18704	CDP-ribitol ribitolphosphotransferase / teichoic acid ribitol-phosphate polymerase	-0.03 (-0.05 to -0.01)	0.041
K01493	dCMP deaminase	-0.03 (-0.05 to -0.01)	0.041
K03856	3-deoxy-7-phosphoheptulonate synthase	-0.03 (-0.05 to -0.01)	0.041
K07738	transcriptional repressor NrdR	-0.03 (-0.05 to -0.01)	0.041
K09735	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.041
K12240	pyochelin synthetase	-0.03 (-0.05 to -0.01)	0.041
K14656	FAD synthetase	-0.03 (-0.05 to -0.01)	0.041
K20859	phosphoribosyl 1,2-cyclic phosphate 1,2-diphosphodiesterase	-0.03 (-0.05 to -0.01)	0.041
K09767	cyclic-di-GMP-binding protein	-0.03 (-0.05 to -0.01)	0.041
K02567	nitrate reductase (cytochrome)	-0.03 (-0.05 to -0.01)	0.041
K03627	putative transcription factor	-0.03 (-0.05 to -0.01)	0.041

K03646	colicin import membrane protein	-0.03 (-0.05 to -0.01)	0.041
K19137	CRISPR-associated protein Csn2	-0.03 (-0.05 to -0.01)	0.041
K01873	valyl-tRNA synthetase	-0.03 (-0.05 to -0.01)	0.041
K01874	methionyl-tRNA synthetase	-0.03 (-0.05 to -0.01)	0.041
K01939	adenylosuccinate synthase	-0.03 (-0.05 to -0.01)	0.041
K03237	translation initiation factor 2 subunit 1	-0.03 (-0.05 to -0.01)	0.041
K03602	exodeoxyribonuclease VII small subunit	-0.03 (-0.05 to -0.01)	0.041
K07572	putative nucleotide binding protein	-0.03 (-0.05 to -0.01)	0.041
K07334	toxin HigB-1	-0.03 (-0.05 to -0.01)	0.042
K01938	formate--tetrahydrofolate ligase	-0.03 (-0.05 to -0.01)	0.042
K04654	hydrogenase expression/formation protein HypD	-0.03 (-0.05 to -0.01)	0.042
K00031	isocitrate dehydrogenase	-0.03 (-0.05 to -0.01)	0.042
K06937	7,8-dihydro-6-hydroxymethylpterin dimethyltransferase	-0.03 (-0.05 to -0.01)	0.042
K00125	formate dehydrogenase (coenzyme F420) beta subunit	-0.03 (-0.05 to -0.01)	0.042
K00891	shikimate kinase	-0.03 (-0.05 to -0.01)	0.042
K01740	O-acetylhomoserine (thiol)-lyase	-0.03 (-0.05 to -0.01)	0.042
K01868	threonyl-tRNA synthetase	-0.03 (-0.05 to -0.01)	0.042
K02551	2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase	-0.03 (-0.05 to -0.01)	0.042
K03282	large conductance mechanosensitive channel	-0.03 (-0.05 to -0.01)	0.042
K04517	prephenate dehydrogenase	-0.03 (-0.05 to -0.01)	0.042
K07076	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.042
K18918	RHH-type transcriptional regulator, rel operon repressor / antitoxin RelB	-0.03 (-0.05 to -0.01)	0.042
K00991	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	-0.03 (-0.05 to -0.01)	0.042
K01170	tRNA-intron endonuclease, archaea type	-0.03 (-0.05 to -0.01)	0.042
K04652	hydrogenase nickel incorporation protein HypB	-0.03 (-0.05 to -0.01)	0.043
K03057	transcription factor S	-0.03 (-0.05 to -0.01)	0.043
K07134	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.043
K01883	cysteinyl-tRNA synthetase	-0.03 (-0.05 to -0.01)	0.044
K00077	2-dehydropantoate 2-reductase	-0.03 (-0.05 to -0.01)	0.044
K00443	coenzyme F420 hydrogenase subunit gamma	-0.03 (-0.05 to -0.01)	0.044
K01579	aspartate 1-decarboxylase	-0.03 (-0.05 to -0.01)	0.044
K01755	argininosuccinate lyase	-0.03 (-0.05 to -0.01)	0.044
K02323	DNA polymerase II small subunit	-0.03 (-0.05 to -0.01)	0.044
K05934	precorrin-3B C17-methyltransferase	-0.03 (-0.05 to -0.01)	0.044
K09141	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.044
K11717	cysteine desulfurase / selenocysteine lyase	-0.03 (-0.05 to -0.01)	0.044
K09724	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.044
K01104	protein-tyrosine phosphatase	-0.03 (-0.05 to -0.01)	0.044
K03055	DNA-directed RNA polymerase subunit K	-0.03 (-0.05 to -0.01)	0.044
K03687	molecular chaperone GrpE	-0.03 (-0.05 to -0.01)	0.044
K01155	type II restriction enzyme	-0.03 (-0.05 to -0.01)	0.044
K03421	methyl-coenzyme M reductase subunit C	-0.03 (-0.05 to -0.01)	0.044

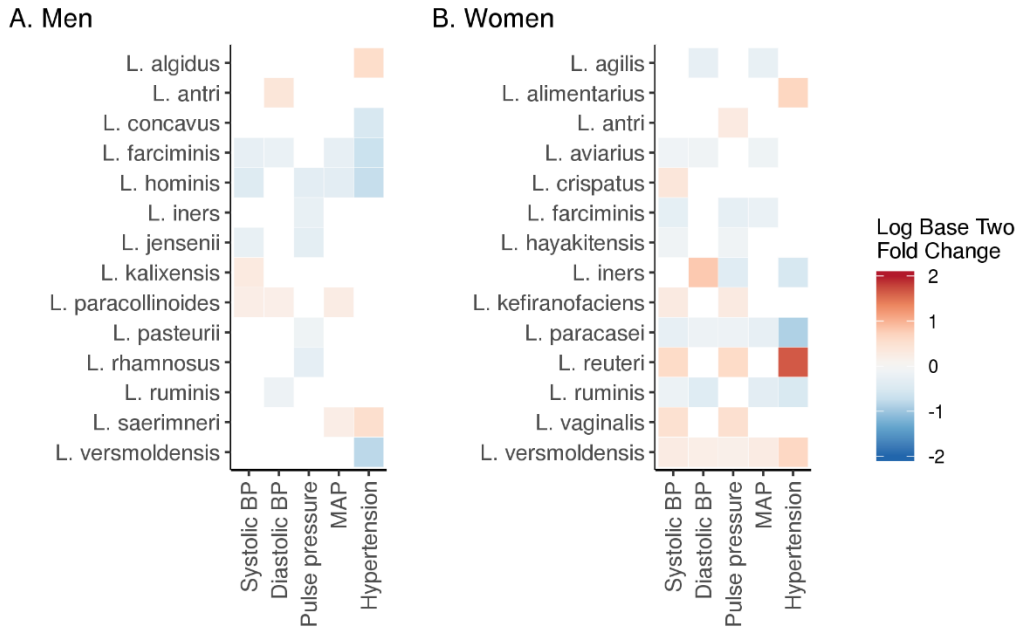
K19545	lincosamide nucleotidyltransferase A/C/D/E	-0.03 (-0.05 to -0.01)	0.044
K00793	riboflavin synthase	-0.03 (-0.05 to -0.01)	0.045
K04766	acetoin utilization protein AcuA	-0.03 (-0.05 to -0.01)	0.045
K08972	putative membrane protein	-0.03 (-0.05 to -0.01)	0.045
K04043	molecular chaperone DnaK	-0.03 (-0.05 to -0.01)	0.045
K11131	H/ACA ribonucleoprotein complex subunit 4	-0.03 (-0.05 to -0.01)	0.045
K01265	methionyl aminopeptidase	-0.03 (-0.05 to -0.01)	0.045
K02492	glutamyl-tRNA reductase	-0.03 (-0.05 to -0.01)	0.045
K02988	small subunit ribosomal protein S5	-0.03 (-0.05 to -0.01)	0.045
K03310	alanine or glycine:cation symporter, AGCS family	-0.03 (-0.05 to -0.01)	0.045
K03534	L-rhamnose mutarotase	-0.03 (-0.05 to -0.01)	0.045
K03686	molecular chaperone DnaJ	-0.03 (-0.05 to -0.01)	0.045
K06147	ATP-binding cassette, subfamily B, bacterial	-0.03 (-0.05 to -0.01)	0.045
K06862	energy-converting hydrogenase B subunit Q	-0.03 (-0.05 to -0.01)	0.045
K08316	16S rRNA (guanine966-N2)-methyltransferase	-0.03 (-0.05 to -0.01)	0.045
K07271	lipopolysaccharide cholinephosphotransferase	-0.03 (-0.05 to -0.01)	0.045
K09726	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.045
K14128	F420-non-reducing hydrogenase small subunit	-0.03 (-0.05 to -0.01)	0.045
K00600	glycine hydroxymethyltransferase	-0.03 (-0.05 to -0.01)	0.046
K09714	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.046
K00590	site-specific DNA-methyltransferase (cytosine-N4-specific)	-0.03 (-0.05 to -0.01)	0.046
K01866	tyrosyl-tRNA synthetase	-0.03 (-0.05 to -0.01)	0.046
K03637	cyclic pyranopterin monophosphate synthase	-0.03 (-0.05 to -0.01)	0.046
K05896	segregation and condensation protein A	-0.03 (-0.05 to -0.01)	0.046
K07310	Tat-targeted selenate reductase subunit YnfF	-0.03 (-0.05 to -0.01)	0.046
K14113	energy-converting hydrogenase B subunit D	-0.03 (-0.05 to -0.01)	0.046
K14125	energy-converting hydrogenase B subunit P	-0.03 (-0.05 to -0.01)	0.046
K19147	5-methylcytosine-specific restriction enzyme subunit McrC	-0.03 (-0.05 to -0.01)	0.046
K01179	endoglucanase	-0.03 (-0.05 to -0.01)	0.046
K01872	alanyl-tRNA synthetase	-0.03 (-0.05 to -0.01)	0.046
K03976	Cys-tRNA(Pro)/Cys-tRNA(Cys) deacylase	-0.03 (-0.05 to -0.01)	0.046
K06200	carbon starvation protein	-0.03 (-0.05 to -0.01)	0.046
K07030	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.046
K14111	energy-converting hydrogenase B subunit B	-0.03 (-0.05 to -0.01)	0.046
K14116	energy-converting hydrogenase B subunit G	-0.03 (-0.05 to -0.01)	0.046
K14119	energy-converting hydrogenase B subunit J	-0.03 (-0.05 to -0.01)	0.046
K14120	energy-converting hydrogenase B subunit K	-0.03 (-0.05 to -0.01)	0.046
K14122	energy-converting hydrogenase B subunit M	-0.03 (-0.05 to -0.01)	0.046
K14123	energy-converting hydrogenase B subunit N	-0.03 (-0.05 to -0.01)	0.046
K00440	coenzyme F420 hydrogenase subunit alpha	-0.03 (-0.05 to -0.01)	0.046
K02035	peptide/nickel transport system substrate-binding protein	-0.03 (-0.05 to -0.01)	0.046
K02189	cobalt-precorrin 5A hydrolase	-0.03 (-0.05 to -0.01)	0.046
K02866	large subunit ribosomal protein L10e	-0.03 (-0.05 to -0.01)	0.046

K07111	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.046
K20021	L-cysteine desulfidase	-0.03 (-0.05 to -0.01)	0.046
K00244	fumarate reductase flavoprotein subunit	-0.03 (-0.05 to -0.01)	0.046
K03042	DNA-directed RNA polymerase subunit A"	-0.03 (-0.05 to -0.01)	0.047
K06920	7-cyano-7-deazaguanine synthase	-0.03 (-0.05 to -0.01)	0.047
K12297	23S rRNA (guanine2445-N2)-methyltransferase / 23S rRNA (guanine2069-N7)-methyltransferase	-0.03 (-0.05 to -0.01)	0.047
K00219	2,4-dienoyl-CoA reductase (NADPH2)	-0.03 (-0.05 to -0.01)	0.047
K00239	succinate dehydrogenase / fumarate reductase, flavoprotein subunit	-0.03 (-0.05 to -0.01)	0.047
K01425	glutaminase	-0.03 (-0.05 to -0.01)	0.047
K01738	cysteine synthase	-0.03 (-0.05 to -0.01)	0.047
K02906	large subunit ribosomal protein L3	-0.03 (-0.05 to -0.01)	0.047
K04651	hydrogenase nickel incorporation protein HypA/HybF	-0.03 (-0.05 to -0.01)	0.047
K07068	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.047
K09721	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.047
K09732	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.047
K09742	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.047
K10553	fructose transport system permease protein	-0.03 (-0.05 to -0.01)	0.047
K14110	energy-converting hydrogenase B subunit A	-0.03 (-0.05 to -0.01)	0.047
K14112	energy-converting hydrogenase B subunit C	-0.03 (-0.05 to -0.01)	0.047
K14114	energy-converting hydrogenase B subunit E	-0.03 (-0.05 to -0.01)	0.047
K14115	energy-converting hydrogenase B subunit F	-0.03 (-0.05 to -0.01)	0.047
K14121	energy-converting hydrogenase B subunit L	-0.03 (-0.05 to -0.01)	0.047
K14124	energy-converting hydrogenase B subunit O	-0.03 (-0.05 to -0.01)	0.047
K15773	HTH-type transcriptional regulator / antitoxin HipB	-0.03 (-0.05 to -0.01)	0.047
K00558	DNA (cytosine-5)-methyltransferase 1	-0.03 (-0.05 to -0.01)	0.047
K02548	1,4-dihydroxy-2-naphthoate polyprenyltransferase	-0.03 (-0.05 to -0.01)	0.047
K02570	periplasmic nitrate reductase NapD	-0.03 (-0.05 to -0.01)	0.047
K02881	large subunit ribosomal protein L18	-0.03 (-0.05 to -0.01)	0.048
K02907	large subunit ribosomal protein L30	-0.03 (-0.05 to -0.01)	0.048
K03969	phage shock protein A	-0.03 (-0.05 to -0.01)	0.048
K08357	tetrathionate reductase subunit A	-0.03 (-0.05 to -0.01)	0.048
K01520	dUTP pyrophosphatase	-0.03 (-0.05 to -0.01)	0.048
K01547	potassium-transporting ATPase ATP-binding subunit	-0.03 (-0.05 to -0.01)	0.048
K00609	aspartate carbamoyltransferase catalytic subunit	-0.03 (-0.05 to -0.01)	0.048
K01588	5-(carboxyamino)imidazole ribonucleotide mutase	-0.03 (-0.05 to -0.01)	0.048
K03168	DNA topoisomerase I	-0.03 (-0.05 to -0.01)	0.048
K11175	phosphoribosylglycinamide formyltransferase 1	-0.03 (-0.05 to -0.01)	0.048
K03330	glutamyl-tRNA(Gln) amidotransferase subunit E	-0.03 (-0.05 to -0.01)	0.048
K03529	chromosome segregation protein	-0.03 (-0.05 to -0.01)	0.048
K03750	molybdopterin molybdotransferase	-0.03 (-0.05 to -0.01)	0.048
K07812	trimethylamine-N-oxide reductase (cytochrome c)	-0.03 (-0.05 to -0.01)	0.048
K18209	fumarate reductase (CoM/CoB) subunit A	-0.03 (-0.05 to -0.01)	0.048

K07458	DNA mismatch endonuclease, patch repair protein	-0.03 (-0.05 to -0.01)	0.048
K00567	methylated-DNA-[protein]-cysteine S-methyltransferase	-0.03 (-0.05 to -0.01)	0.049
K01011	thiosulfate/3-mercaptopyruvate sulfurtransferase	-0.03 (-0.05 to -0.01)	0.049
K14392	sodium/pantothenate symporter	-0.03 (-0.05 to -0.01)	0.049
K01153	type I restriction enzyme, R subunit	-0.03 (-0.05 to -0.01)	0.049
K01756	adenylosuccinate lyase	-0.03 (-0.05 to -0.01)	0.049
K02982	small subunit ribosomal protein S3	-0.03 (-0.05 to -0.01)	0.049
K03166	DNA topoisomerase VI subunit A	-0.03 (-0.05 to -0.01)	0.049
K07096	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.049
K08298	L-carnitine CoA-transferase	-0.03 (-0.05 to -0.01)	0.049
K09482	glutamyl-tRNA(Gln) amidotransferase subunit D	-0.03 (-0.05 to -0.01)	0.049
K10896	fanconi anemia group M protein	-0.03 (-0.05 to -0.01)	0.049
K11212	LPPG:FO 2-phospho-L-lactate transferase	-0.03 (-0.05 to -0.01)	0.049
K14982	two-component system, OmpR family, sensor histidine kinase CiaH	-0.03 (-0.05 to -0.01)	0.049
K15731	carboxy-terminal domain RNA polymerase II polypeptide A small phosphatase	-0.03 (-0.05 to -0.01)	0.049
K18830	HTH-type transcriptional regulator / antitoxin PezA	-0.03 (-0.05 to -0.01)	0.049
K00763	nicotinate phosphoribosyltransferase	-0.03 (-0.05 to -0.01)	0.049
K12141	hydrogenase-4 component F	-0.03 (-0.05 to -0.01)	0.049
K01546	potassium-transporting ATPase potassium-binding subunit	-0.03 (-0.05 to -0.01)	0.049
K02933	large subunit ribosomal protein L6	-0.03 (-0.05 to -0.01)	0.049
K07442	tRNA (adenine57-N1/adenine58-N1)-methyltransferase catalytic subunit	-0.03 (-0.05 to -0.01)	0.049
K02904	large subunit ribosomal protein L29	-0.03 (-0.05 to -0.01)	0.049
K06913	uncharacterized protein	-0.03 (-0.05 to -0.01)	0.049
K01975	RNA 2',3'-cyclic 3'-phosphodiesterase	-0.03 (-0.05 to -0.01)	0.049
K02994	small subunit ribosomal protein S8	-0.03 (-0.05 to -0.01)	0.049
K06019	pyrophosphatase PpaX	-0.03 (-0.05 to -0.01)	0.050
K07114	Ca-activated chloride channel homolog	-0.03 (-0.05 to -0.01)	0.050

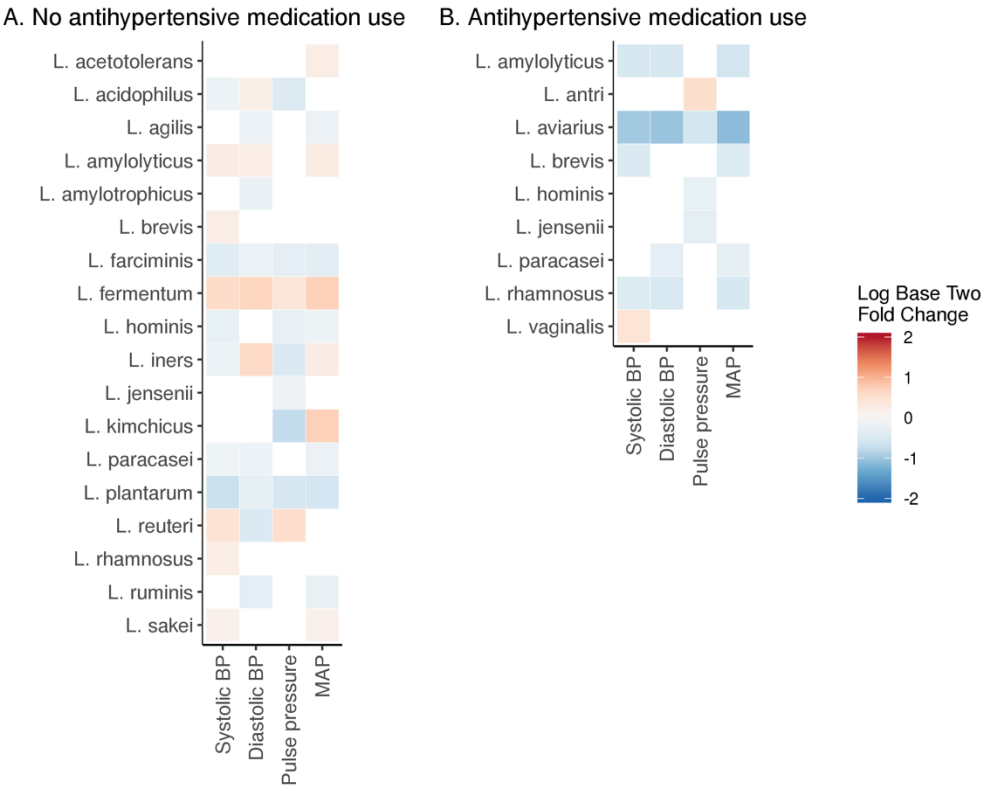
P values are adjusted for multiple testing using FDR correction.

Figure S1. Associations between *Lactobacilli* and blood pressure by sex.



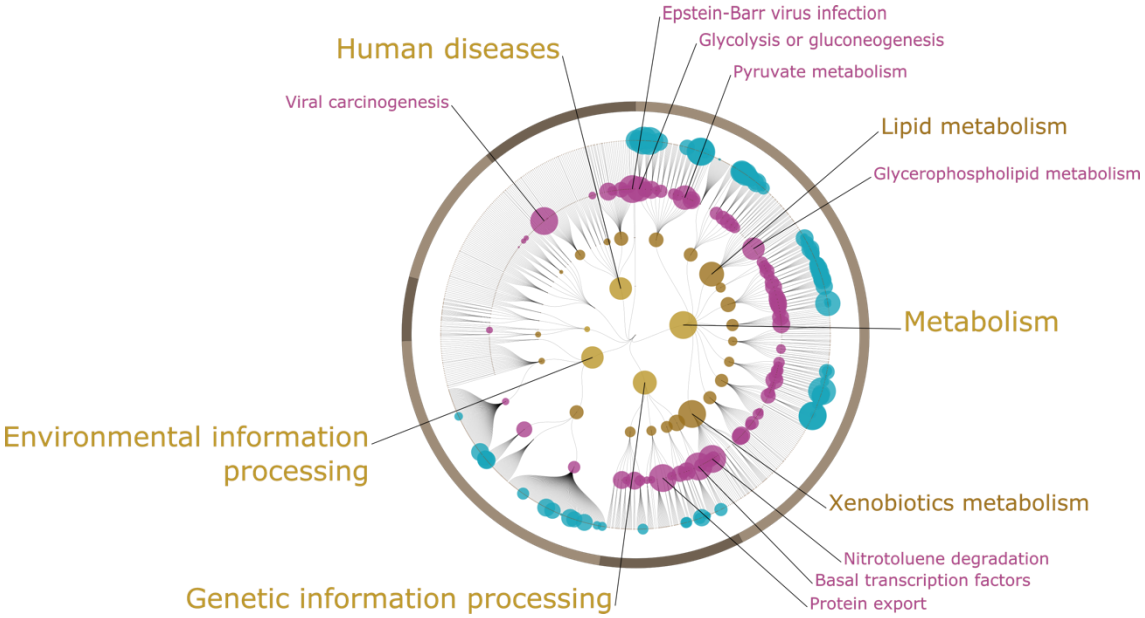
The models are adjusted for age, BMI, smoking, exercise, diuretics, beta blockers, calcium channel blockers, and renin–angiotensin system blockers. N=3 819 for women and N=3 134 for men. BP, Blood pressure; MAP, Mean arterial pressure.

Figure S2. Associations between *Lactobacilli* and blood pressure by antihypertensive medication use.



The models are adjusted for age, sex, BMI, smoking, exercise, diuretics, beta blockers, calcium channel blockers, and renin–angiotensin system blockers. Association with bacterial plasmid is denoted using asterisk. N=1 253 for antihypertensive medication users and N=5 700 for non-users. BP, Blood pressure; MAP, Mean arterial pressure.

Figure S3. Functional pathways associated with systolic BP.



For the module (cyan), pathway (purple), biological process (dark brown), and biological category (light brown) functional layers, node size corresponds to the average inverse P value of the KO group assigned to that node. Only KO groups that were negatively associated with systolic BP were included. Node titles are shown for nodes in the three highest layers with a size > 200.